CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 1 APRIL, 1941 NUMBER 4

The Occurrence and Pathology of Spontaneous Carcinoma of the Lung in Mice

H. Gideon Wells, M.D., Maud Slye, Sc.D., and Harriet F. Holmes

(From the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago, Chicago, Illinois)

(Received for publication March 10, 1941)

Mice share with men the peculiarity of having tumors of the lung among the most common neoplasms. However, the lung tumors of mice are very different from the common cancer of the lung in man. The latter arises in the bronchi, leads to occlusion of the bronchi with its sequelae of atelectasis, abscess formation, unresolved pneumonia, fibrosis, and empyema, and is almost unexcelled in the extent of its widespread metastasis. The tumors of the lung of mice arise in the subpleural regions from the alveolar epithelium, and invading the bronchi in the form of papillary ingrowths do not often lead to the widespread necrosis and suppuration common to human lung cancer.

Of particular importance is the absence of metastasis. In the recent series of papers on the production of experimental lung tumors no mention whatever can be found of the occurrence of metastases, with the exception of papers by Campbell and by Magnus. Campbell (2) produced lung tumors by dusting mice with road dust, and of 26 cases thus produced there were metastases in three—one in the heart and two in the kidney. He comments on the fact that these were the first metastases to be observed in England, and also that this was the first case of metastasis to the heart. In a later paper (3) he reports that in 10 mice with tumors of the lung induced by extract of cardboard, one tumor gave rise to metastases in the mediastinal nodes and pleura. Magnus (5) produced tumors of the lung by giving 1,2,5,6-dibenzanthracene by stomach tube to mice, and observed one metastasis to the liver and one to the suprarenal. These are all the cases of induced lung tumors with metastases that we can find.

Of metastases in spontaneous tumors, the record seems equally blank, for no metastases are recorded in the great number of spontaneous tumors observed, except in a paper published from this laboratory in 1914 (6). Here were reported the spontaneous occurrence of 160 lung tumors observed in 6,000 mice, of which 4 showed metastasis outside the lung. This was the first, and until recently the only, example of metastases of lung tumors, and served to establish them as true neoplasms. Since that time we have observed many more instances of lung tumors, both with and without metastases.

Of 147,132 mice coming to autopsy in the Slye laboratory, where every mouse is allowed to live out its span of life, 2,865 mice had lung tumors, or about 2 per cent. Since a considerable proportion of the mice do not live to tumor age, the figure of mice of tumor age would be raised to about 3 per cent. Of these lung tumors, 104 showed metastases outside the lungs, or 3.6 per cent. All these showed metastases in the mediastinal lymph nodes, many of them also showed growths along the chest wall and on the diaphragm. Only 10 showed metastases to other locations; namely, 5 in the kidney, 3 in the heart (Fig. 1), 1 in the seminal vesicle, and 1 in the skull.

There were also 284 tumors that were unquestionably malignant, as shown by metastases throughout the lung, making a total of 388 malignant growths, or 13.6 per cent of the lung tumors. Of the rest, a considerable number were probably malignant, as shown by invasion of the bronchi, and it was difficult to decide whether some were multicentric or metastatic and so were included as multicentric benign growths. The experimental study of lung cancer has shown that the growth begins in the alveolar walls, frequently extends into the bronchi, and presents no sharp distinction between malignancy and benignancy. There is equal difficulty in distinguishing between benign and malignant spontaneous tumors. Sometimes tumors that would be considered benign on the basis of structure and lack of invasive character are found to give rise to metastasis, while other tumors of wild growth fail to produce metastatic growth. All observers comment on the infrequency of mitotic figures, and the lack of this sort of evidence of malignancy.

Furthermore, when present, mitotic figures are not indicative of malignancy.

In Tyzzer's articles (7, 8) he speaks of "several cases" of epidermoid cancer of the lung in his series. In our large series but 7 distinctly presented this character.

With reference to the origin of these tumors in the alveolar walls, which is well discussed by Grady and Stewart (4), and the question whether these cells are mesenchymal or epithelial, the behavior of the metastatic tumors may throw some light. For of the 104 cases with metastasis, no less than 33 of the metastatic growths showed more or less the structure of a sarcoma (Fig. 2) even when the primary growth exhibited no sarcomatous elements, although in not a few primary growths this sarcomatous structure was apparent (Fig. 3). No primary sarcomas of the lung, without epithelial elements, have been observed in our mice.

Campbell has directed attention to this phenomenon of sarcoma-like transition in primary lung tumors of mice in the following words, "The tumors are regarded as malignant if they give rise to metastases, and also if, without metastases, *they contain infiltrating spindle cells* or spheroidal cancer cells," and shows pictures of the spindle cell growth. This is a true spindle cell growth with collagen fibers between the cells, and not the oat cell growth of undifferentiated lung carcinoma. It is interesting to consider that Andervont (1) reports that 4 pulmonary tumors induced in mice by dibenzanthracene, and one which arose spontaneously, when transplanted into the subcutaneous tissues became changed to spindle cell sarcomas, although the parent tumors were definitely adenomatous. Apparently when the cancer cells get away from the alveolar walls which serve as a framework and perhaps maintain their epithelial character, they assume a spindle cell character resembling sarcoma. Perhaps this may be looked upon as evidence of their mesenchymal nature.

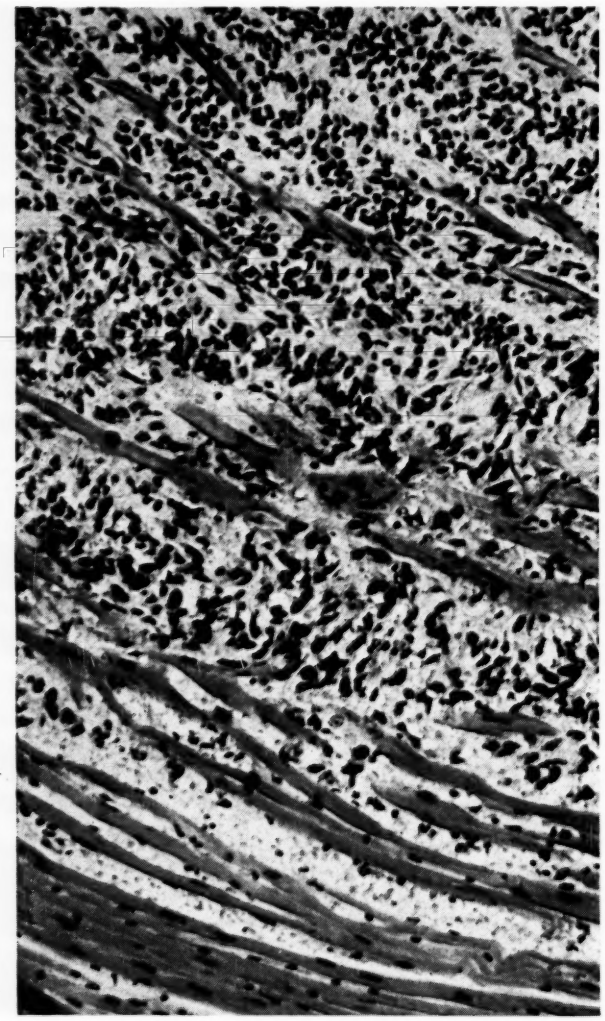


FIG. 1.—Metastasis of carcinoma of the lung in the heart. × 235.

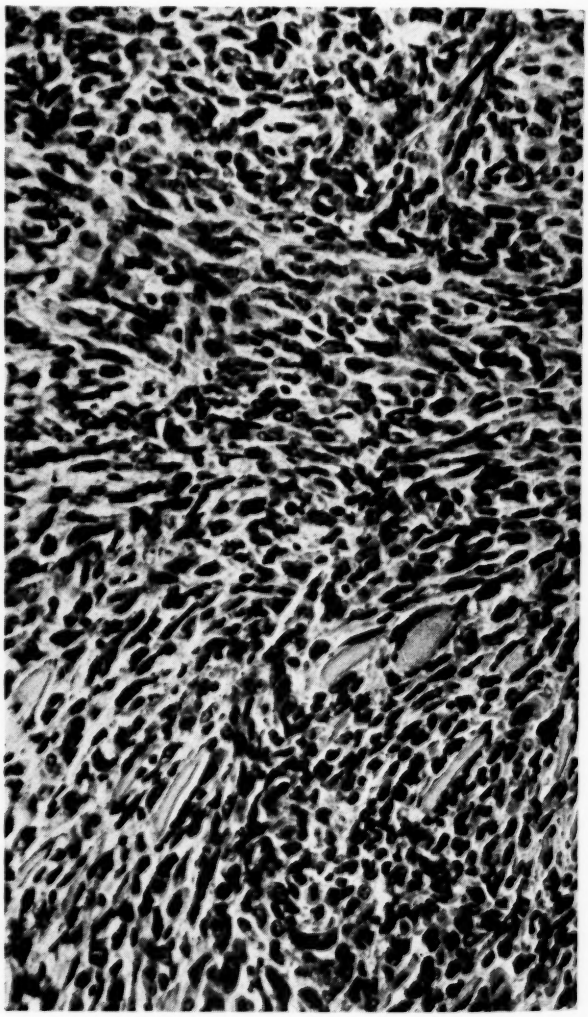


FIG. 2.—Spindle cell metastasis in chest wall of primary carcinoma of the lung of a mouse. × 280.

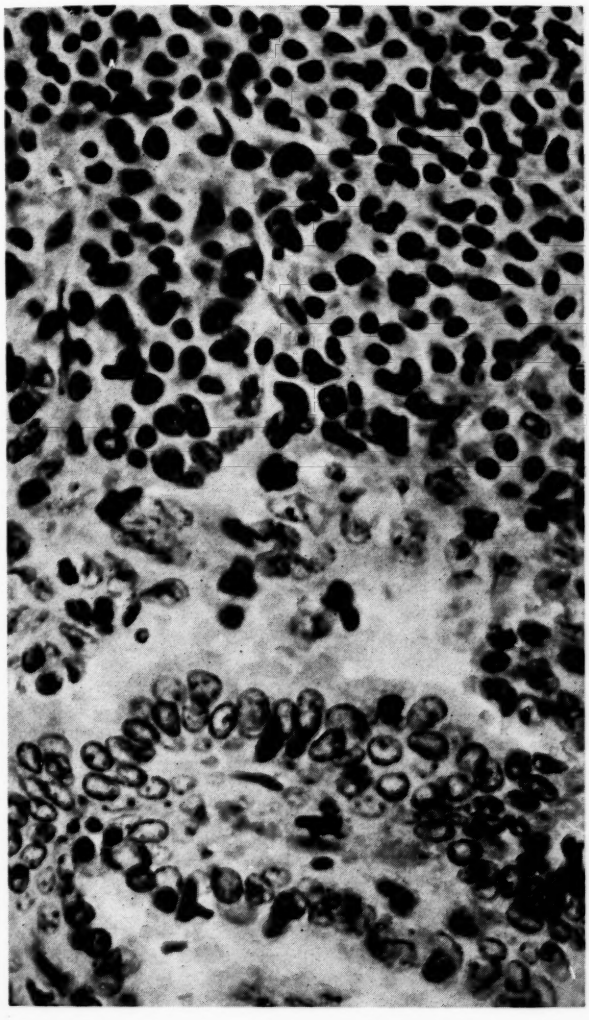


FIG. 3.—Sarcoma-like area in primary carcinoma of the lung of a mouse. × 565.

SUMMARY AND CONCLUSIONS

Of 147,132 mice coming to autopsy in the Slye laboratory, where every mouse is allowed to live out its span of life, 2,865 mice (2 per cent) had lung tumors. Of these lung tumors, 104 (3.6 per cent) showed metastases outside the lungs. All of these showed metastases in the mediastinal lymph nodes; many also showed growths along the chest wall and on the diaphragm. Only 10 showed metastases to other organs; namely, 5 in the kidney, 3 in the heart, 1 in the seminal vesicle, and 1 in the skull.

Of the 104 cases with metastases, 33 of the metastatic growths showed more or less the structure of a sarcoma. This was true even when no sarcomatous elements were seen in the primary growth. Although in a number of primary lung tumors this sarcomatous structure was apparent, no primary sarcomas of the lung, without epithelial elements, have been observed in these mice.

The mesenchymal or epithelial origin of these lung tumors is discussed.

REFERENCES

1. ANDERVONT, H. B. Pulmonary Tumors in Mice. VII. Further Studies on the Serial Transmission of Lung Tumors Occurring in Inbred Mice. Pub. Health Rep., **54**:1519-1524. 1939.
2. CAMPBELL, J. A. Effects of Road Dust "Freed" from Tar Products upon Incidence of Primary Lung-Tumours of Mice. Brit. J. Exper. Path., **18**:215-223. 1937.
3. CAMPBELL, J. A. Carcinogenic Agents Present in Atmosphere and Incidence of Primary Lung Tumours in Mice. Brit. J. Exper. Path., **20**:122-132. 1939.
4. GRADY, H. G., and H. L. STEWART. Histogenesis of Induced Pulmonary Tumors in Strain A Mice. Am. J. Path., **16**:417-432. 1940.
5. MAGNUS, H. A. Experimental Production of Malignant Papillomata of Lung in Mice with 1:2:5:6-Dibenzanthracene. J. Path. & Bact., **49**:21-31. 1939.
6. SLYE, M., H. F. HOLMES, and H. G. WELLS The Primary Spontaneous Tumors of the Lungs in Mice. Studies on the Incidence and Inheritability of Spontaneous Tumors in Mice. J. M. Research, **30**:417-442. 1914.
7. TYZZER, E. E. A Series of Spontaneous Tumors in Mice with Observations on the Influence of Heredity on the Frequency of their Occurrence. J. M. Research, **21**:479-518. 1909.
8. TYZZER, E. E. A Series of Twenty Spontaneous Tumors in Mice, with the Accompanying Pathological Changes and the Results of the Inoculation of Certain of these Tumors into Normal Mice. J. M. Research, **17**:155-197. 1907.

The Production of Tumors by Tobacco Tars

Curtis M. Flory, M.D.

(From the Department of Pathology of the University of Chicago, Chicago, Illinois)

(Received for publication March 12, 1941)

The main purpose of this paper is to report the results of an investigation of the carcinogenic effects of destructive distillates of tobacco and of pipe tar. It is concerned chiefly with the experimental production of benign and malignant tumors in rabbits, rats, and mice painted or injected with tobacco tars. To give the background, a review will be presented of some of the clinical studies which have indicated that the smoking of tobacco is a factor in the etiology of carcinoma of the lip, the tongue and oral cavity, the pharynx and larynx, and the lung in man. Experimental studies of the carcinogenic action of various tars will also be reviewed.

Carcinoma of the lip, formerly referred to as "pipe-smoker's cancer," has been said by Ewing (12) to be traceable in many cases to the irritation of a pipe or cigar, or both. Broders (4) found that three-fourths of the smokers with this disease smoked pipes. Other factors, such as sunlight, are also etiologically important.

Almost all of Bloodgood's (2) patients with carcinoma of the tongue used tobacco. Abbe (1) showed that all save one of 90 men with oral cancer were heavy smokers. In 1,413 patients with cancer of the palate, tongue, mouth, and lung, Hoffman (16) found a higher percentage of heavy smokers than in a similar number of controls. He (17) stated that pipe smoking has a direct relationship to cancer of the lip and tongue, and concluded that smoking increases the liability to cancer of the mouth, throat, esophagus, larynx, and lung. Ewing (12) has written that tobacco has "a predominant influence in the development of cancer of the buccal mucosa." He included tobacco as a contributing factor in cancer of the larynx, esophagus, and lung.

The increased use of cigarettes and the habit of inhaling smoke deep into the lungs, has been blamed by many for the increased incidence of carcinoma of the lung. Müller (28) found a high percentage of heavy smokers among males with primary carcinoma of the lung. Lickint (23, 24) stated that smoking was the chief cause of carcinoma of the lung. Simonds (51) placed tobacco smoke as among the possible causes of this tumor, but did not consider it an important etiological factor.

It is not unlikely that a carcinogenic hydrocarbon could be formed during the smoking of tobacco. During smoking tobacco is subjected first to a mild destructive distillation process, then to combustion at temperatures of from 370-700° C. (11). Carcinogenic substances have been formed from other organic materials by similar processes at similar temperatures. Extracts of coal soots are carcinogenic (8). Kennaway (18) has found slight carcinogenic activity in tars produced by the destructive distillation of coal at temperatures as low as 450° C. Tars produced from coal heated to the temperature found in a briar pipe (11), 560° C., were almost as carcinogenic as those produced at 1,250° C. Tars from acetylene heated to 700° C., and isoprene heated to 720° C., the upper limit of pipe temperatures, were carcinogenic (18). At higher temperatures the destructive distillates of petroleum, human skin, and yeast (18), cholesterol (19), turpentine (56), rice polishings and crude sugar (20), all were shown to be carcinogenic.

Tobacco, like many of the above substances, is a complex mixture of chemicals, and by subjecting it to combustion and destructive distillation processes many workers have attempted to obtain carcinogenic substances.

In Table I are listed the attempts to produce carcinomas with tobacco products. The later work of Roffo and that of Sugiura are discussed separately.

In 1936 Roffo (34, 35) reported the results of painting the destructive distillates of Kentucky tobacco daily on the ears of rabbits. The watery 0-120° C. fraction produced no tumors in 10 months. The 120-350° C. fraction, a tar, produced papillomas in most animals in 7 months, and squamous cell carcinomas in all in 9 months. The residual tar also produced carcinomas, but not as frequently. Spectroscopy suggested the presence of 1,2-benzanthracene. However, further spectroscopic work (36) on this and other tobacco tars showed only the presence of condensed benzenic nuclei.

Roffo (37) obtained similar results with Egyptian and Turkish tobaccos. He (38, 39, 43) subsequently used 9 varieties of tobacco, painting the rabbits every other day with a 120-350° C. destructive distillate. The first papilloma was seen in 26 days, and many tumors developed between 40 and 50 days. Each of the tars produced tumors in most of the animals. Many of these tumors seem to have been papillomas, but he stated that 10 rabbits painted with tar from Egyptian tobacco developed squamous cell carcinomas in from 8 to 14 months.

In 1939 Roffo (40, 41) reported spectroscopic studies of the destructive distillates of tobacco distilled at 380° C. A similarity was found in the absorption bands of tobacco tar and of 1,2-benzpyrene between the ranges of 2100 A. and 3100 A. His conclusion that the distillate contained 1,2-benzpyrene would be more understandable if he had demonstrated strong bands with their lower edges at 4040 A. and 4270 A. It is in this region of the spectrum that Hieger (14) and others (26) have located the bands of 3,4-benzpyrene. (Formerly numbered 1,2-benzpyrene (19).)

Recently Roffo (42) prepared tars by the combustion of tobacco in a large pipe. He condensed the smoke in water and in cotton filters, extracted the tar, and painted rabbits' ears every other day. The first papilloma was seen at 49 days, and 8 rabbits developed tumors by 290 days. At 12 months each rabbit had from 6 to 13 tumors, some of which were described as squamous cell carcinomas.

In 1940 Sugiura (52) heated leaf tobaccos in an iron still at from 100-500° C., then at from 500-900° C., collecting the products of each distillation. Groups of mice were painted twice weekly with the distillates. Only 1 mouse developed a squamous cell carcinoma. This appeared on a mouse painted for 315 days with the 500-900° C. tar. Using an oily mixture of both distillates he painted the ears of rats for periods up to 65 weeks without effect, and also painted the ears of rabbits 3 times a week for from 52 to 95 weeks without tumor production.

TABLE I: ATTEMPTS TO PRODUCE CARCINOMAS WITH TOBACCO PRODUCTS

Year	Author	Substance used	Animals	Method	Time *	Results
1900	Brosch (5)	Tobacco juice	Guinea pigs	Painting	?	Proliferation
1911	Wacker and Schmincke (54, 55).	Pipe tars	Rabbits	Injection	21 D	Proliferation
1923	Hoffman, Schreus and Zurhelle (15).	Tobacco tar denicotinized.	Mice	Painting	14 D	Hairlessness
1928	Helwig (13)	Pipe tar	Rabbits	Injection	4 M	Proliferation
		Nicotine free ether extract of pipe tar.	Mice	Painting	1 Y	Benign ulcers
		400-500° C. destructive distillate of tobacco.	Mice	Painting	8 M	Benign ulcers
1930	Roffo (29)	Tobacco smoke	Rabbit	Blowing smoke	.....	Oral leukoplakia.
	Roffo (30, 31)	Condensed tobacco smoke in water.	Rabbit	Painting	8 M	Papillary carcinoma on ear
1931	Chickamatsu (9)	Tobacco tar	Rabbit	Painting	225 D	Cancroid of ear
1931	Cooper, Lamb, and Sanders (11).	Tar from pipe smoke, cold, hot, and in various solvents.	Mice	Painting	16 M	1 carcinoma
1932	Roffo (33, 32)	Jet of tobacco smoke	Rabbits	Smoke on ear	3 Y	1 carcinoma with cervical metastases
	Roffo (34)	Pills of tobacco tar	Rats	Implants	8 M	Neoplasm-like tumors
	Moratti (27)	Cigars	Dog	Inserted in gall bladder.	.....	Chronic inflammation
		Nicotine solution	Dog	Injected in breast.	.....	Chronic inflammation
	Bogen and Loomis (3)	Tar from the destructive distillation of tobacco, denicotinized.	Mice	Painting	1 Y	No effect
			Rabbits	Painting	6 M	No effect
	McNally (25)	Tobacco tar	Rats	.....	.....	Too toxic
		Water solution of cigarette smoke.	Rats	Spraying	100 D	No effect
1934	Lu-Fu-Hua (21, 22)	Tar from burning tobacco on 1 ear, plus some coal tar on other ear.	Rabbits	Painting	148-182 D	3 cancers on tobacco ear
		Tobacco tar plus injected cholesterol.	Rabbits	Painting	205 D	1 cancer
1935	Schürch and Winterstein (47).	Denicotinized tar from cigar smoke.	Mice	Painting	258-635 D	No tumors
		Distillates of above cigar tar.	Mice	Painting	60-480 D	No tumors
		Cigar tar	Rabbits	Painting ears.	223 D	No effect
				Painting mouth	548 D	No effect
		Coal tar to one ear until warts appear, ear amputated. Other ear painted with cigar smoke tar.	Rabbits	Painting	918 D	Carcinoma in 1 rabbit at 344 D, warts in 6 at 918 D
1937	Schürch and Winterstein (48).	As above	Rabbits	Painting	48 M	Carcinoma in 1 rabbit, warts in 2
1936	Campbell (7, 8)	Cigarette smoke tar	Mice	Painting	16 M	1 carcinoma
1937	Taki (See Kinoshita) (20).	Tar from tobacco pipes	Mice	Painting	130 D	2 carcinomas; 3 papillomas

* D = days; M = months; Y = years.

Experimental

Materials and Methods

Tobacco used.—The tobacco used was a cheap grade of cured Kentucky tobacco. The entire leaf and leaf stem were used.

Temperature determinations.—Tobacco smoked slowly in briar pipes burned at from 425-560° C. A few puffs raised the temperature above 600° C., and on several occasions almost to 700° C. Temperatures of 645-704° C. were measured in clay pipes during puffs, and on one occasion reached 750° C. During puffs cigarettes burned at from 604-670° C. Matches burned at from 850° to almost 1000° C. These findings agree with those of Cooper, Lamb, and Sanders (11).

All temperatures were measured by alumel-chromel thermocouples, made of number 28 wire, and calibrated against a standardized alumel-chromel thermocouple. The determinations were accurate to within 12° C.

Preparation of the destructive distillates of tobacco.—The destructive distillates were prepared in an electrically heated steel still, shown in Fig. 1.

The still was filled with 2.5 kg. of dry tobacco; the asbestos gasket, soaked in water-glass (liquid sodium silicate), put in place; and the lid fastened tightly so as to prevent the escape of fumes. The still was then slowly heated to 700° C. Six to 8 hours were required for a distillation. A straight-tubed water-cooled condenser leading into a filter flask was used to collect the water and tar, and the noncondensable products were led into a hood.

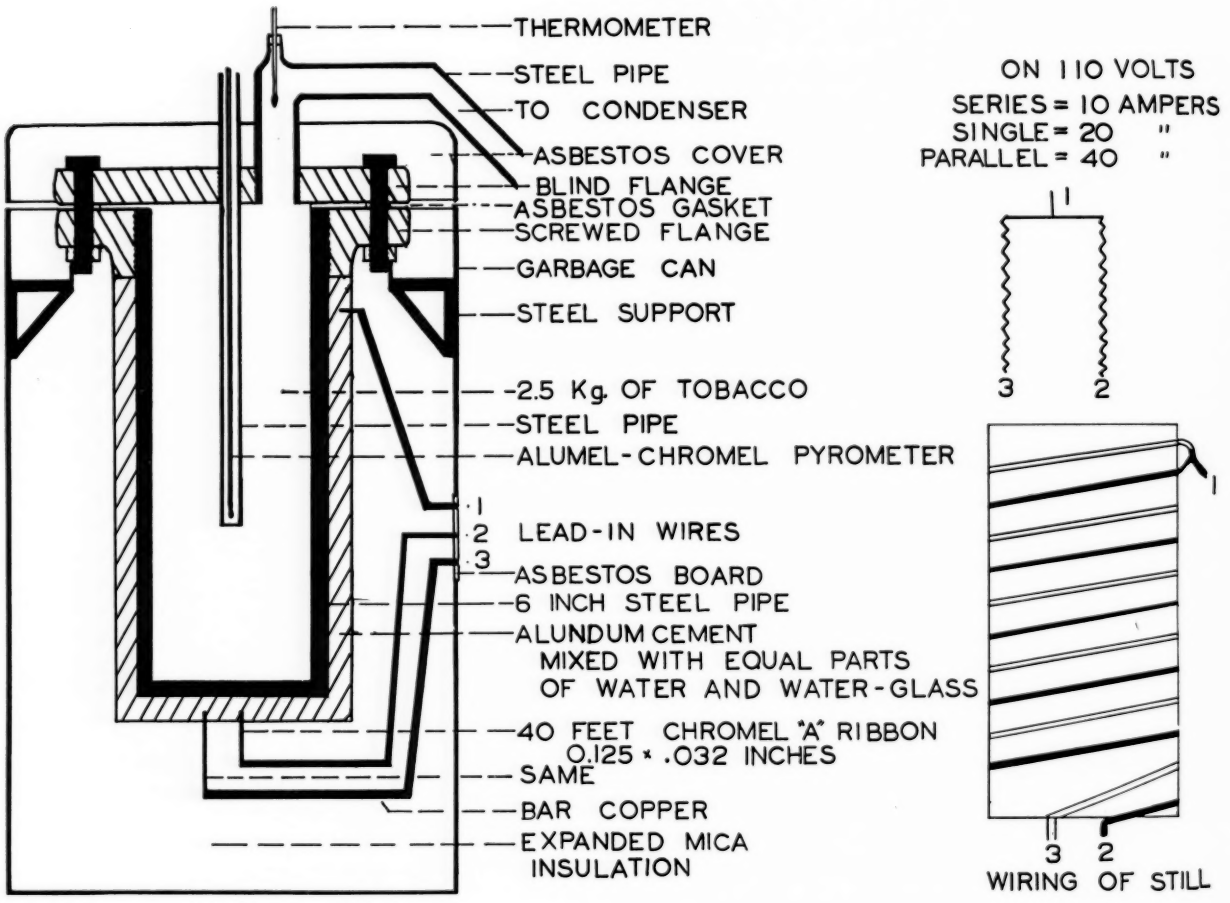


Fig. 1.—The electrically heated still used in preparing the destructive distillates of tobacco.

The 0-120° C. fraction was taken when a thermometer in the still outlet reached 120° C. This fraction consisted largely of water. Any oily material which came over was added to the next fraction. The second, the 120-350° C. fraction, included all condensable material obtained before an alumel-chromel pyrometer, placed in the center of the tobacco in the still, registered 350° C. This fraction consisted of approximately equal layers of an oily black liquid and a heavier, brownish, watery liquid. This mixture was reheated and all material boiling below 120° C. removed. The remaining black, homogenous, tarry substance is called the 120-350° C. destructive distillate tar. The third fraction was taken from 350-700° C. as measured by the pyrometer. This distillate consisted

of thick tarry material and a little water which was removed in a separatory funnel. All residue in the condenser was added to this tar, which is called the 350-700° C. destructive distillate tar.

For each kg. of tobacco distilled, the 0-120° C. fraction yielded 3 gm. of tar (which were added to the 120-350° C. fraction) and 131 gm. of watery material; the 120-350° C. fraction yielded 90.2 gm. of tarry material, and 95 gm. of watery material boiling under 120° C.; and the 350-700° fraction contained 27.5 gm. of tar, and 8.4 gm. of watery material. The amount of the 120-350° tar was over 3 times as great as that of the 350-700° C. tar.

Preparation of pipe tar.—Tobacco was coarsely ground and burned in small clay pipes. The pipe stems were passed through small corks, and these inserted in the ends of 6 small steel pipes threaded into the side of a larger steel pipe. From one end of this large pipe the smoke passed down through water in a partly filled liter flask, through a liter cylinder filled with glass wool, and then through a second flask containing water. Continuous suction was furnished by a water pump. The pipes were lighted with wooden matches. Six pipes were usually burned at one time. In this apparatus the tobacco burned at the temperatures found in the briar and clay pipes during puffs, from 520-730° C. as measured by a thermocouple.

After from 1 to 2 kg. of tobacco were burned, the apparatus, the pipes excepted, was freed of tar by washing first with water, then with ether. The combined water in the apparatus and the water and ether from the washings were evaporated at reduced pressure at 70° C. A thin tar was obtained, called pipe tar, parts of which solidified at room temperature. An average of 113 gm. of tar was obtained from 1 kg. of tobacco. This tar is probably similar, at least qualitatively, to what could be obtained from ordinary pipe smoke.

Animals and methods.—Rabbits, mice, and rats were used as test animals. The rabbits were mostly domestic albinos, and were obtained from three different dealers. No difference in the reactions of these rabbits to tobacco tars could be attributed to their strain or color. The mice were all white. They were not of an inbred strain, but were bred from a small colony in this laboratory. No spontaneous squamous cell carcinomas have been seen in about 2,000 of these mice, some observed in other experiments for as long as 2 years. The rats were albinos, from a stock bred in this laboratory.

All mice and rabbits were painted 5 times a week, the mice on the back, the rabbits on the inner surface of the right ear. In all rabbits where tumors were studied histologically the subauricular lymph nodes and lungs were similarly examined, and frequently other viscera as well. Multiple sections were made of all subauricular nodes.

Certain mice were painted with a denicotinized tar. The crude tar was shaken twice with equal parts of 2 per cent HCl solution, draining off the watery liquid each time, then washed of the excess acid with an equal part of distilled water. This treated tar is called denicotinized tar.

Control studies with coal tar.—No extensive work was done on rabbits to determine the time of production of carcinomas by coal tar. However a few animals were painted with a coal tar of known carcinogenic potency in order to obtain tar tumors for histological comparisons. The inner surfaces of the right ears of 4 rabbits were painted 5 times a week with coal tar dissolved in equal parts of benzene. Papillomas first appeared in from 42 to 159 days. One rabbit was killed at 188 days, the rest at 525 days. Nineteen tumors were examined histologically, and all were benign papillomas.

Fifteen mice were painted on the back of the neck 5 times a week with the same benzene solution of coal tar. The first papilloma was seen at 107 days, and the first squamous cell carcinoma of the skin at 126 days. In all, 13 mice developed these carcinomas, the last at 256 days. Three of these squamous cell carcinomas metastasized to the lungs, another to a lymph node. Obviously the mice used in these experiments were relatively susceptible to tar painting.

Classification of rabbit tumors.—In this work the rabbit tumors have been classified as papillomas and carcinomatoid tumors. The papillomas are the same kind of wart-like growths Yamagiwa and Ichikawa (57) called "folliculo-epitheliomata," and Rous and Kidd (44) common papillomas.

The carcinomatoid tumors require more explanation. Rous and Kidd (44) divided coal tar-induced rabbit tumors into four groups: common papillomas, frill horns, carcinoids, and carcinomas. No frill horns were noted in our animals. They described carcinoids as tumors which had the morphology of squamous cell carcinomas, yet vanished or assumed a benign form when tarring was left off. At first these tumors were markedly invasive, with strands of individual cells growing down into the connective tissue. Grossly they were "raised, raw, ragged disks with infiltrating edges . . . . or else raised discoid ulcers." Occasionally they penetrated the lacunae in the cartilages to the other side of the ear. Frequently strands of cells grew directly into the local lymphatics, yet Rous and Kidd never observed a metastasis to a distant site. With continued tarring these tumors eventually took on gross forms like those of old papillomas, or else retrogressed. When tarring was stopped the carcinoids apparently retrogressed or became papillomas. All attempts to transplant carcinoids failed.

Recently (45) Rous and Kidd have suggested that these tumors be called carcinomatoids to avoid confusion with the argentaffine tumors of the human in-

testinal tract. In this paper the term carcinomatoid has accordingly been used.

Extreme anaplasia of a locally invasive tumor may raise the question of malignancy, but not establish it beyond question. The only true criterion for a carcinoma of the ear of a rabbit is a metatasis to a lymph node or other distant site, or a tumor that is transplantable. Ulceration, local destruction of the ear, and local lymphatic vessels may all occur in carcinomatoid tumors.

Table II: Grams of Tar Painted on Each Rabbit

Tars	Per painting: First 70 days	Per painting: First year	Total for year
350-700° C. destructive distillate tar	0.38	0.56	146.0
120-350° C. destructive distillate tar	0.34	0.38	98.5
Pipe tar	0.31	0.32	84.0

Effects of tobacco tars on rabbits.—The 350-700° C. destructive distillate tar, the 120-350° C. destructive distillate tar, and the pipe tar were each painted 5 times a week on the inner surfaces of the right ears of rabbits. The quantities of the tars used are shown in Table II.

The increase in quantity of the 350-700° C. tar painted was required to cover the papillomas which appeared.

Production of papillomas.—The 350-700° C. tar caused rapid thickening of the skin at the painting site, and after the first week thin sheets of loose skin could be peeled from the ears. From then on the ears were peeled of loose skin about twice a week to permit direct application of the tar to the skin. After 30 days hyperkeratosis of the hair follicles was noted, and rapidly increased. The last of the hair disappeared at about this time.

The ears of the rabbits painted with the other 2 tars showed similar, but less marked changes.

The earliest time at which a papilloma was seen was on the 37th day in a rabbit painted with pipe tar. The mean time of appearance of the papillomas was, however, the best guide to the papilloma-producing action of the tars, and this mean time was 57 days for the 350-700° C. tar, 75 days for the 120-350° C. tar, and 108 days for the pipe tar. After the appearance of the papillomas drawings were made of the ears at monthly intervals to make records of the time of appearance, number, and character of the tumors. In Tables III, IV, and V are shown the number of rabbits painted with each tar, their color, the days on which the rabbits first developed tumors, the increase in number and kinds of tumors examined histologically, and the present status of the rabbits. Table VIII is a summary of all the tumors produced in rabbits.

As can be seen from these tables, the 350-700° C. tar produced the most tumors. The average rabbit painted with this tar developed a maximum of 9 papillomas. The 120-350° C. tar produced an average of only 4.3 tumors, and the pipe tar 4.7 tumors per rabbit.

Despite this greater average production of tumors by the 350-700° C. tar, it is evident from the tables that there was great variation in papilloma production among the rabbits of each series. Some rabbits in each series developed a large number of papillomas, some few or none. It is evident that the variation of susceptibility of the individual animals was greater than the difference in potency of the tars.

The size and shape of the papillomas also varied. Some of the papillomas appeared as low warts 6 or 8 mm. across, growing rapidly into large stalked tumors 2 or 3 cm. in diameter and height. Others never grew over a few mm. high and retained a broad base.

The early papillomas were composed largely of thick hyperkeratotic epithelium projecting up from the ear in filiform processes. As the tumors became larger the

Table III: Production of Tumors by the 350-700° C. Destructive Distillate Tobacco Tar

Rabbit	Color	Tumor first seen, days	Number of tumors (mostly papillomas) seen; R = Regression of all tumors; Days: 50	100	150	200	250	300	350	400	450	500	550	600	Tumors examined histologically: Papillomas	Carcinomatoids	Status of animals at indicated days
21	White	49	5	7	8	8	6	6	10	12	8	8	8	6	6	1	Alive 660
22	White	50	3	6	7	5	5	..	..	..	..	..	..	..	7	..	Died 265
23	White	72	..	3	5	1	..	..	..	..	..	..	..	..	1	..	Died 238
24	White	64	..	6	13	12	12	..	..	..	..	..	..	..	15	..	Died 272
25	White	49	3	6	11	11	11	9	13	14	14	8	..	..	3	1	Died 590
26	White	72	..	4	4	7	8	6	7	12	11	11	..	..	7	..	Died 540
27	White	66	..	2	3	4	4	..	..	..	..	..	..	..	4	..	Died 268
28	White	57	..	8	6	7	8	9	10	7	9	3	3	..	2	.	Died 585
29	White	57	..	5	3	7	6	6	7	11	10	6	..	..	1	2	Died 534
30	White	49	..	2	8	13	8	9	9	16	11	10	..	..	19	1	Died 521
31	Black-white	74	..	2	2	1	2	1	R	R	R	R	R	R	..	..	Alive 660
32	Black-white	79	..	2	4	4	5	..	..	..	..	..	..	..	3	..	Died 289
	Total														68	5	

TABLE IV: PRODUCTION OF TUMORS BY THE 120-350° C. DESTRUCTIVE DISTILLATE TOBACCO TAR

Rabbit number	Color	Tumor first seen, days	Number of tumors (mostly papillomas) seen R = Regression of all tumors — Days 50	100	150	200	250	300	350	400	450	500	550	600	Tumors examined histologically: Papillomas	Carcinomatoids	Status of animals at indicated days
1	White	93	..	1	1	2	2	4	4	5	7	9	6	..	4	..	Died 555
2	White	63	..	6	..	..	..	..	..	..	..	..	..	..	6	2	Died 127
3	White	68	..	3	5	4	4	7	6	5	..	..	..	..	1	..	Died 439
4	White	190	..	..	..	1	2	2	1	2	..	..	..	..	2	..	Died 422
5	White	50	1	1	2	2	4	..	..	..	..	..	..	..	3	..	Died 299
6	White	73	..	3	3	2	2	2	2	..	..	..	..	..	1	3	Died 376
7	White	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Died 23
8	White	68	..	1	1	3	3	1	..	..	..	..	..	..	1	..	Died 321
9	White	89	..	2	R	1	1	R	1	1	3	2	..	..	4	..	Died 510
10	White	75	..	5	5	6	6	7	6	11	11	8	9	8	5	..	Died 625
11	White	50	1	2	1	R	1	1	3	4	10	11	..	..	3	..	Died 528
12	White	137	..	..	3	2	3	2	3	2	..	..	..	..	3	..	Died 434
13	Black-white	142	..	..	1	R	R	..	..	..	..	..	..	..	..	..	Died 286
14	White	344	..	..	..	..	..	..	1	2	2	R	1	R	..	..	Alive 660
15	Black-white	117	..	..	3	..	..	..	..	..	..	..	..	..	1	..	Died 150
16	White	73	..	2	1	4	..	..	..	..	..	..	..	..	6	..	Died 264
17	Grey	104	..	..	1	1	1	R	2	1	1	..	..	..	1	..	Died 497
Total															40	5	

TABLE V: PRODUCTION OF TUMORS BY PIPE TAR

Rabbit number	Color	Tumor first seen, days	Number of tumors (mostly papillomas) seen R = Regression of all tumors — Days 50	100	150	200	250	300	350	400	450	500	550	600	Tumors examined histologically: Papillomas	Carcinomatoids	Status of animals at indicated days
Series I																	
41	Grey	62	..	2	..	..	..	..	..	..	..	..	..	..	1	1	Died 135
42	Grey	..	..	.	..	.	..	..	..	..	..	..	..	..	.	.	Died 95
43	Grey	49	1	1	1	2	..	..	..	..	..	..	..	..	2	..	Died 211
44	White	37	1	R				..	..		..	..	..	..	1	..	Died 132
45	White	111	..	.	1	1	2	1	1	R					1	..	Died 411
46	White	305	..			.	..	..	1	1	1	1	1	1	1	..	Alive 640
47	White	52	..	1	R	R	R	1	1	5	4	5	4	2	2	..	Alive 640
48	White	113	..	..	1	2	2	1	1	2	4	4	4	5	2	..	Alive 640
49	White	374	..	..	.	.		..	..	1	2	3	4	3	1	..	Alive 640
50	Tan	111	..	..	3	9	10	7	8	12	16	11	9	12	4	..	Alive 640
51	White	119	..	..	..	..	..	..	..	..	..	..	..	..	1	..	Died 131
52	White	132	..	..	1	1	1	2	4	2	1	2	6	5	4	..	Alive 640
53	White	94	..	1	1	1	1	3	5	7	6	5	4	5	3	..	Alive 640
Series II																	
54	White	188	..	..	..	1	..	..	..	..	..	..	..	..	1	1	Died 232
55	White	247	..	..	..		1	2	2	4	3	..	..	..	1	..	Alive 500
56	White	107	..		1	R	2	1	1	1	..	..	..	..	1	..	Killed 418
91	White	89	..	1	2	3	4	4	5	4	..	..	..	..	1	..	Alive 440
92	White	99	..	1	2	2	5	7	8	10	..	..	..	..	1	..	Alive 440
93	White	99	..	1	5	5	9	8	9	10	..	..	..	..	1	..	Alive 440
94	White	136	..		2	2	2	3	5	7	..	..	..	..	2	..	Alive 440
95	White	55	..	5	6	9	10	11	6	9	..	..	..	..	2	..	Alive 440
96	White	82	..	2	7	7	9	9	11	11	..	..	..	..	2	..	Alive 440
97	White	.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Killed 357
98	White	136	..	..	2	1	1	R	3	..	..	..	..	.	1	..	Died 398
Total															36	2	

connective tissue was more abundant, and the papillomas became structures with stalks of loose vascular connective tissue and large connective tissue heads covered by irregular hyperkeratotid epithelium (Fig. 2). No invasiveness of the epithelium was seen.

Regression of the papillomas.—Cachexia usually caused rapid regression of the large papillomas, but in many rabbits regressive tendencies were seen without apparent cause. As can be seen from the tables the regressions were most frequent in the animals painted with the 120-350° C. destructive distillate. Some regressions were seen in the rabbits painted with pipe tar.

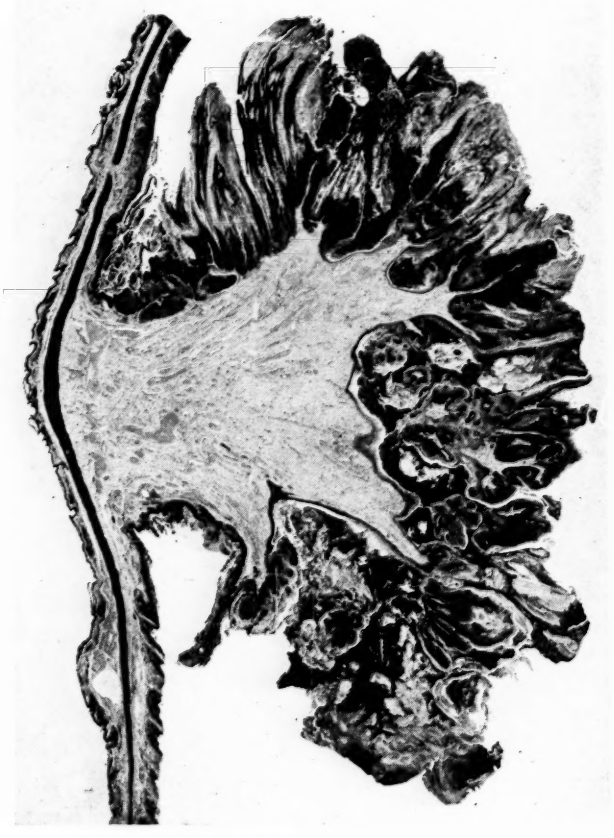


FIG. 2.—A papilloma on the ear of a rabbit (No. 43) painted with pipe tar for 211 days. Mag. × 6.

In 6 rabbits painted with the 350-700° C. tar, however, regressive tendencies were seen after about 350 days. Histologically the regressing papillomas showed much dense collagenous tissue in their stalks, and sections taken elsewhere in the ears showed similar fibrosis between the epithelium and the cartilage. In the end stage of regression only low nubbins of tissue were seen grossly. Histologically these were small mounds of dense fibrous tissue covered by smooth but hyperkeratotic epithelium. Fibrosis of the stalk was a prominent histological feature in the regression of these tumors.

Cause of the regression of papillomas in rabbits painted with 350-700° C. destructive distillate tar.—Something besides cachexia seemed necessary to account for the late regression of so many papillomas in these 6 rabbits painted with the 350-700° C. tar. The potency of the destructive distillate was roughly the same, as 3 white rabbits (not included in Table III) developed papillomas in 59, 59, and 84 days. A fourth killed on the 84th day had no tumors.

In the rabbits in which regressions of tumors were taking place there did not seem to be any systemic resistance to further papilloma formation, as in 4 of the 6 rabbits in which regressions were occurring on the inside of the right ear, small papillomas were appearing where the tar was accidentally rubbed on the backs of the right and left ears.

This lack of systemic resistance was confirmed by painting the insides of the left ears of 2 of these rabbits with this tar from the 540th day on, the painting of the right ears being continued. Multiple papillomas appeared on the inside of the left ear of rabbit No. 21 on the 42nd day, and of rabbit No. 31 on the 62nd day (not included in Table III), although in rabbit No. 21 the tumors on the right ear continued to regress and in rabbit No. 31 the right ear remained without tumors despite the continued painting.

It appears probable that the regression of the papillomas, when not caused by cachexia, is caused by some local change in the papillomas themselves accompanied by resistance of the surrounding skin to further papilloma formation. It seems probable that this is due to fibrosis, and not to a general resistance of the rabbit to the papilloma stimulating action of the tar.

Production of carcinomatoid tumors.—Out of a total of 156 tumors examined histologically in the rabbits painted with tobacco tars, 12 were carcinomatoid tumors, the rest papillomas.

A typical example of these tumors was one seen in a biopsy of the ear of rabbit No. 30 painted with 350-700° C. tar. This tumor was removed on the 229th day along with 1 large and 4 small papillomas. This carcinomatoid was a low rounded tumor 7 mm. across and 2.5 mm. high. The time of its appearance was unknown. Histologically the epithelium on the surface of the tumor was hyperkeratotic, and thin strands of epithelium projected down into the connective tissue (Fig. 3). On serial sections groups of cells lay free in the connective tissue and others were growing in lymphatic vessels (Fig. 4). All these cells were well keratinized and not anaplastic or hyperchromatic. This carcinomatoid was probably completely excised. The subauricular node, removed at the same time, was normal histologically. At death at 521 days only 8 papillomas and 5 regressed papillomas were found. The subauricular lymph node and lungs were normal.

Three other similar carcinomatoid tumors were found on other rabbits painted with 350-700° C. tar.

One tumor was biopsied on rabbit No. 29, on the 485th day, and grew slowly during the last 49 days of life. Both the first specimen and the tumor removed at death showed local intralymphatic growth. A small carcinomatoid tumor was found on the ear of rabbit No. 25 at death at 593 days. Its tumor cells had formed many epithelial pearls which were encased by dense connective tissue. Some well keratinized cells had invaded local lymphatics. The subauricular node and lungs contained no tumor cells.

Rabbit No. 21 is still living. A biopsy at 650 days showed a carcinomatoid tumor with local lymphatic invasion. This tumor has been growing slowly for 90 days. The subauricular node is not enlarged.

Only one of the carcinomatoid tumors examined was anaplastic. A second carcinomatoid tumor was discovered on rabbit No. 29 at the time of the animal's death. It had first appeared as a papilloma near the tip of the ear at 167 days. It remained as a papilloma for about 200 days, then seemed to regress during the last 70 days. At autopsy the tumor was a firm rounded nubbin 1 cm. in diameter and grossly looked much like the regressed papillomas described above. On section the tumor was 1 cm. long and 6 mm. thick (Fig. 5). It was composed of moderately anaplastic squamous cells. These were slightly hyperchromatic, with deep staining nuclei and pale eosinophilic cytoplasm. A few mitotic figures were found. The cells, in small nests and threads, formed many small epithelial pearls. The tumor apparently arose on the inner side of the ear and extended around the tip to the other side of the ear. The tumor was focally ulcerated. The tumor cells had invaded the connective tissue more extensively than most carcinomatoids; many tumor cells lay just above the cartilage, and in one place grew through a small defect in the cartilage. Near one margin the tumor cells had extended under intact epithelium, and also into the connective tissue and the lymphatic vessels about a large vein and several nerves (Fig. 6). This

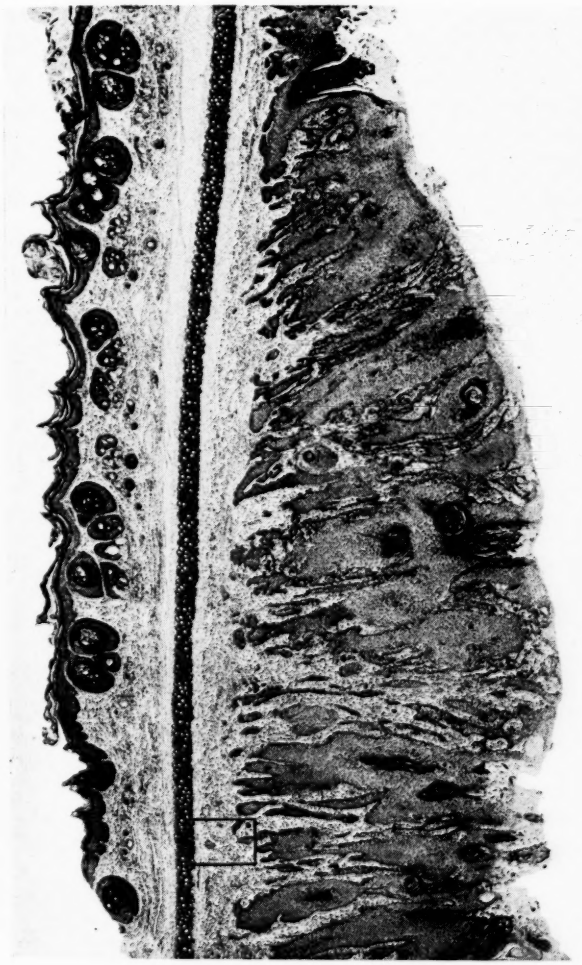


FIG. 3.—A carcinomatoid tumor on the ear of a rabbit (No. 30) painted with the 350-700° C. destructive distillate tar of tobacco for 229 days. Mag. × 22.

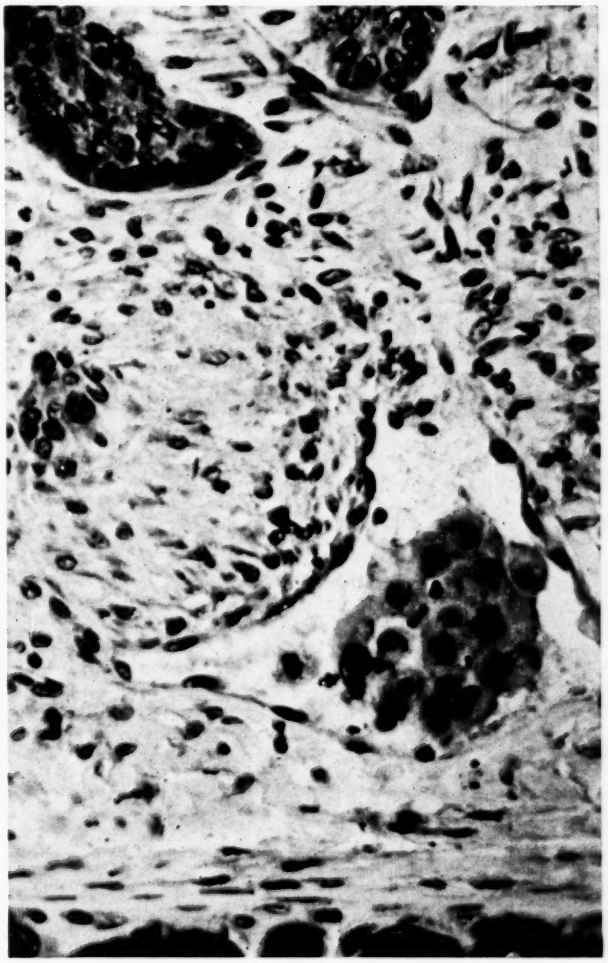


FIG. 4.—A higher magnification of the small area in Fig. 3 enclosed by a rectangle, showing a group of squamous epithelial cells in a lymphatic vessel. Serial sections showed this to be a cord of tumor cells. Mag. × 375.

tumor had the local appearance of a squamous cell carcinoma, but has been classified as a carcinomatoid tumor since no metastases were found in either the subauricular lymph node or lungs.

Five carcinomatoid tumors were found in the rabbits painted with the 120-350° C. destructive distillate tar. Two of these were found on rabbit No. 2, which died on the 127th day. Six benign papillomas and 2 carcinomatoids were found. Both were typical infiltrative flat tumors, and both showed local invasion of the lymphatic spaces. The subauricular node and lungs contained no tumor.

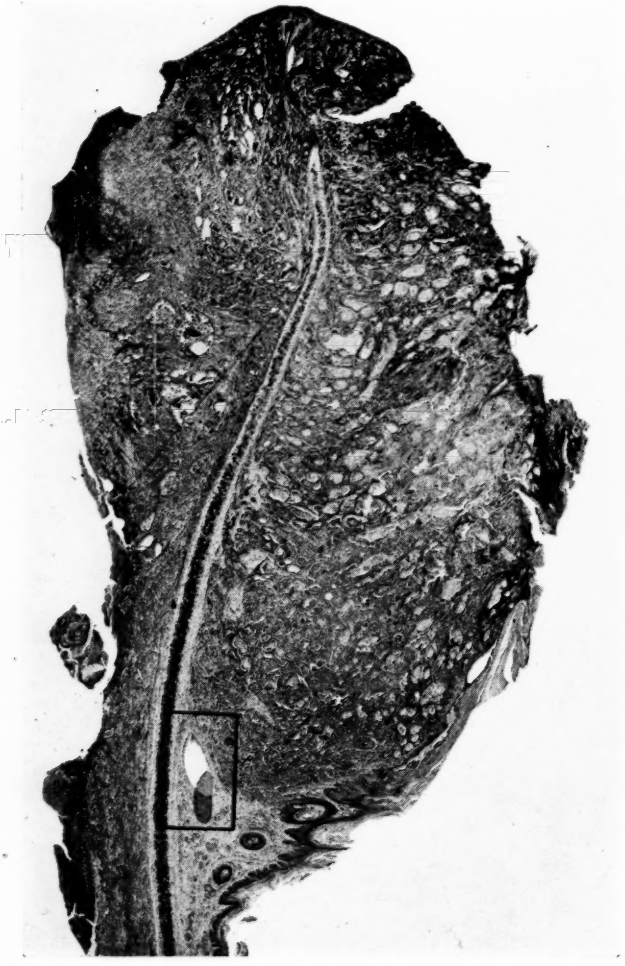


FIG. 5.—An anaplastic carcinomatoid tumor on the ear of a rabbit (No. 29) painted with the 350-700° C. destructive distillate tar of tobacco for 534 days. The small circular areas are epithelial pearls. Note the growth of the tumor along the cartilage plate and also the growth under intact epithelium. Mag. × 7.

What was probably a tumor embolus was found in the lymph node of a rabbit painted with this 120-350° C. tar. Rabbit No. 6 died on the 376th day. One of the tumors was a small papilloma, the other 3 flat warty growths measuring 10, 8, and 8 mm. in diameter. These 3 growths had been present for 220 days and had grown very little, remaining as low small tumors. Histologically all were carcinomatoids. One showed infiltration of epithelial cells into the connective tissue in threads and clusters of cells, the other 2 showed a more invasive tendency, with intralymphatic growth of tumor cells. These last 2 tumors also showed more epithelial pearls than the first. All 3 were superficially ulcerated. The lungs contained no tumor cells. In two sections of the subauricular lymph node, lying in one of the lymph sinuses entering the node, was a cluster of what probably were keratinized epithelial cells. The nuclei were large and pale, the cytoplasm was very eosinophilic, and the cell borders were sharp. No intracellular bridges could be made out. Serial sections showed no similar cell clusters, and no such groups of cells have been seen in any of the other lymph nodes studied. If this was, as seems probable, a group of tumor cells, it must be considered as an embolus to the lymph node and not as a definite metastasis.

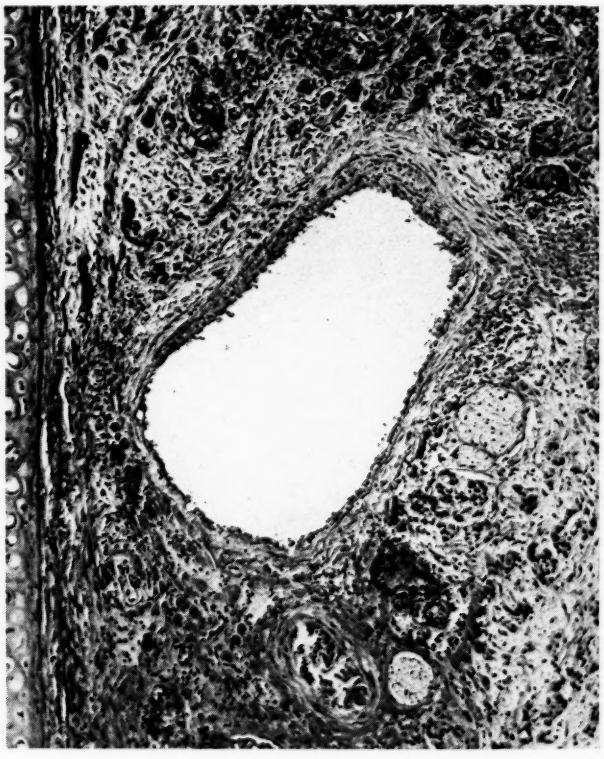


FIG. 6.—A higher magnification of an area similar to that enclosed by a rectangle in Fig. 5, but from another section of the same tumor. The tumor cells are invading the connective tissue and lymphatics about the large vein and the nerves. Mag. × 90.

Rabbit No. 41, painted with pipe tar, died after 135 days with 2 tumors, one a small papilloma, the other a carcinomatoid tumor 3 mm. across showing definite invasiveness. No metastases were found. This tumor

had been present for 73 days as a flat tumor, which not only did not grow, but even became smaller before death.

The second carcinomatoid in the rabbits painted with pipe tar was found on rabbit No. 54 at death after 232 days, along with a papilloma. This carcinomatoid tumor was also small, with local lymphatic invasion (Fig. 7). The subauricular lymph node and

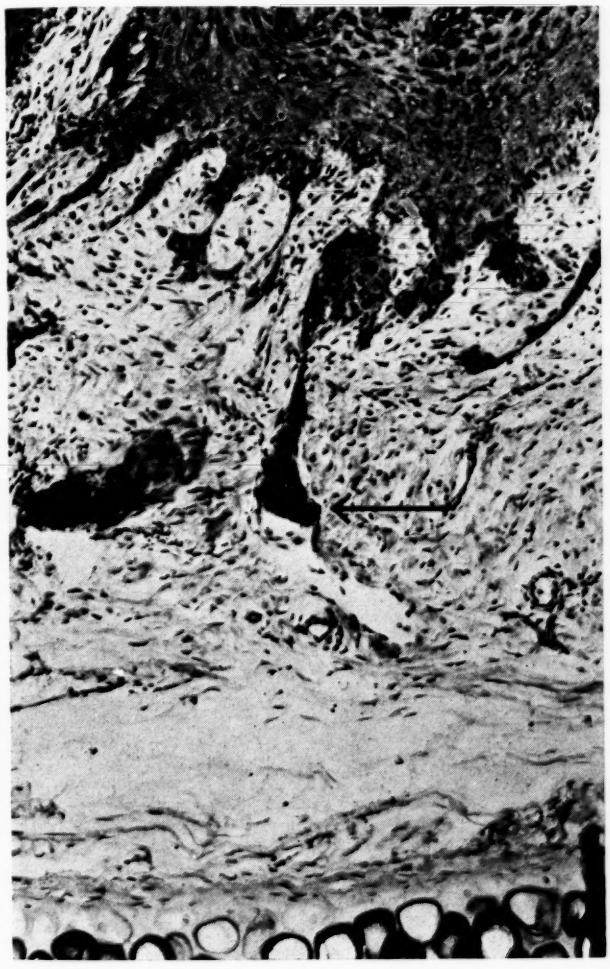


FIG. 7.—A portion of a carcinomatoid tumor produced on a rabbit (No. 54) by painting pipe tar for 232 days. The arrow points to a group of tumor cells growing in a local lymphatic. Mag. × 145.

lungs contained no tumor cells. This carcinomatoid had grown very slowly for 44 days.

Twenty-nine biopsies have been made of all the possibly carcinomatoid tumors on the ears of the 14 rabbits painted with pipe tar that are still alive. Although these animals had been painted for from 425 to 629 days, all of the biopsied tumors were papillomas.

Five carcinomatoid tumors were seen in rabbits painted with fractional distillates, and are discussed under that heading.

A total of 17 of these locally invasive carcinomatoid tumors was seen in 11 rabbits. The first of these was found after 127 days of painting. Another which subsequently proved to be a carcinomatoid probably arose on the 62nd day. Many arose before 200 days, but some as late as 600 days. None of the tumors grew rapidly, most slowly. In 8 out of 11 rabbits the epithelial cells of the tumors invaded the local lymphatics. Out of the 11 rabbits, 10 have died and no distant metasases have been found, although what was probably a tumor embolus to a lymph node was found.

Causes of death.—Of the rabbits painted with the 350-700° C. destructive distillate tar, 10 rabbits died after varying periods of cachexia. Six had pneumonia with abscesses, 2 pyemia, 1 urinary tract infection, and 1 paralysis. No visceral lesions attributable to the tar were observed.

The 120-350° C. tar often caused convulsive symptoms if painted too freely. Ten of the 16 rabbits died a few hours after painting, apparently from acute nicotine poisoning. Two others died of pneumonia, 2 of diarrhea, and 2 of cachexia without focal lesions.

TABLE VI: FRACTIONAL DISTILLATES OF THE 350-700° C. DESTRUCTIVE DISTILLATE TAR AT 10 TO 13 MM. HG. PRESSURE

Temperature of liquid in still	Quantity	Color	Consistency
30-100° C.	60.3 gm.	Yellow	Watery
100-140° C.	77.9 gm.	Brown	Oily
140-180° C.	84.4 gm.	Deep brown	Viscid
180-220° C.	80.2 gm.	Deep brown	Semisolid
220-240° C.	59.3 gm.	Brown-black	Semisolid
Residue	181.3 gm.	Black	Solid tar

Fourteen of the rabbits painted with pipe tar are still alive and being painted. Of the 10 that have died, 5 died acutely of what probably was nicotine poisoning, 1 of pneumonia, 1 of an unknown debilitating disease, and 2, Nos. 56 and 97, were killed.

Fractional distillates of 350-700° C. tar; preparation of distillates.—Since it seemed probable that some chemical substance was the cause of the papilloma-producing action, a fractional distillation was done on the pooled 350-700° C. destructive distillates of 20 kg. of tobacco, 625 gm. of tar in all.

Water and the lower alcohols were first removed by heating to 120° C. in a glycerine bath at atmospheric pressure, leaving 567 gm. of tar. This tar was heated in a glass still at a pressure of from 10 to 13 mm. Hg over first a glycerine, then a solder bath. The quantities obtained are shown in Table VI.

Effect of fractions of tar on rabbits.—Each of 5 fractions and the residue was tested. Two cubic centimeters of benzene were added as a solvent to each gram of substance. All rabbits used were white. All except 2 of the rabbits were painted for at least 186, and most for 200 days. One in the 140-180° C. series

died at 96 days and one in the residue series died at 147 days, both without tumors. The results are shown in Table VII.

It was apparent that the 220-240° C. substance was the most active fraction, but this was only a crude estimate, and the important fact was that the active fraction boiled above about 200° C. at a pressure of 10 to 12 mm. Hg. It was evident that the lower boiling substances were not active.

Table VII: Tumors Produced by Fractional Distillates of 350-700° C. Destructive Distillate Tar

Fraction at 10-13 mm. Hg	Total rabbits	Rabbits with tumors	Time of development in days	Total tumors seen
30-100° C.	4	0		0
30-100° C.	4	0	.....	0
100-140° C.	4	0	.....	0
140-180° C.	5	0	.....	0
180-220° C.	4	1	136	1
220-240° C.	4	4	34, 52, 83, 93	27
Residue.	4	3	41, 117, 171	9

Table VIII: Summary of Tumors Produced on Rabbits by Tobacco Tars

Substance used	Number rabbits	Per cent developing tumors	Mean time, in days	Av. no. per ear	No. tumors studied histologically	Papillomas	Carcinomatoids
350-700° C. destructive distillate tar	12	100	57	9.0	73	68	5
120-350° C. destructive distillate tar	17	94	75	4.3	45	40	5
Pipe tar	24	92	108	4.7	38	36	2
Fractions of the 350-700° C. tar	..	..	..	..	27	22	5
Total					183	166	17

Types of tumors produced.—Of 27 tumors examined histologically 22 were papillomas, and 5 carcinomatoids. Two of the carcinomatoid tumors were seen in rabbits painted with the residue. One appeared after 165 days and was only 3 mm. across when the animal was killed on the 195th day. The other was an 8 mm. carcinomatoid found among 7 papillomas in a rabbit dying after 508 days. Neither carcinomatoid tumor had invaded lymphatics, and the subauricular nodes and lungs of these rabbits showed no tumor.

The other 3 carcinomatoid tumors were in a rabbit painted with the 220-240° C. fraction and killed at 224 days. One of these tumors appeared on the 92nd day and grew very slowly. At death it was only 5 mm. across and 2 mm. high. The other 2 appeared later, and also grew slowly. Two of the 3 showed local lymphatic invasion, but the subauricular nodes and lungs were normal.

Attempt to demonstrate a virus in the papillomas.—The method described by Shope (50) was used, and 2 filtrates made, one from papillomas on rabbits in the 350-700° C. series, the other from papillomas on rabbits in the pipe tar series. One filtrate was inoculated on a scarified strip on the right side of the abdomen of 5 white rabbits, the other on the left side. Other rabbits were injected intramuscularly and intravenously with the filtrates. No tumors developed, and all animals were killed after 60 days.

Effect of injection of the 350-700° C. destructive distillate tar into rats.—One group of white rats was injected intrascapularly with 0.05 cc. of the crude tar. Thirteen survived the immediate toxic effects, and 6 lived 6 months, 2 lived 12 months, and 1 lived 18 months. Another group of rats was similarly injected with 1 cc. of denicotinized tar. Seventeen rats survived the immediate effects, 15 lived 6 months, and 10 are alive at 12 months.

No tumors have been seen in either group. In those which have died, black pigment and oily material in macrophages were seen at the injection site.

Effects of the tobacco tars on mice.—All mice were painted on the back 5 times a week. The 350-700° C. destructive distillate tar was not very toxic and was painted on mice both in the original form used on rabbits, and after denicotinization. Both the 120° C. and 350° C. destructive distillate tar and the pipe tar had to be denicotinized before being painted on mice, as in their original form they caused convulsions and death in most mice.

Table IX: Tumors Produced on Mice by Tobacco Tars

Substance used	Grams per mouse per painting	Number of mice alive Months 0	3	6	9	12	15	18	Papillomas No.	Papillomas Time, in months	Carcinomas No.	Carcinomas Time, in months
350-700° C. destructive distillate tar	0.015	30	29	27	15	7	1	..	4	7.5 to 11.5	..	..
Denicotinized 350-700° C. destructive distillate tar	0.018	46	44	30	8	2	..	..	8	7.5 to 11	1	8.5
Denicotinized 120-350° C. destructive distillate tar	0.015	76	68	55	24	17	5	4	1	14	..	..
Mixture of watery parts of distillates	0.20	30	30	30	26	19	11	1	..	.....	..	..
Denicotinized pipe tar	0.013	60	58	53	40	32	28	20	2	14	1	17

A group of mice were painted with a mixture composed of equal parts of the watery portions of the 0-120° C., the 120-350° C., and the 350-700° C. destructive distillates.

The details of the experiments are shown in Table IX.

The destructive distillate tars produced baldness after a few paintings, and eventually both the pipe tar and the watery mixture from the distillates caused partial baldness.

FIG. 8.—The squamous cell carcinoma produced on a mouse by painting the denicotinized 350-700° C. destructive distillate tar of tobacco for 300 days. The tumor is rather anaplastic, with cells growing in sheets and cords, but not forming typical epithelial pearls. A metastasis to an adjacent lymph node is well keratinized. Mag. × 75.

Papillomas were produced frequently by the 350-700° C. tars. All papillomas were verified histologically. Frequently inflammatory hyperplasias were mistaken grossly for papillomas.

The denicotinized 350-700° C. destructive distillate tar produced a squamous cell carcinoma in a mouse at 8.5 months, at a time when 14 mice were alive in this series. This carcinoma arose at the painting site and grew rapidly for the month before the animal was killed. Histologically the tumor was typical (Fig. 8) and metastasized to an adjacent lymph node. This denicotinized tar seemed to be more toxic than the crude 350-700° C. tar, but produced more papillomas and the only malignancy.

The denicotinized pipe tar also produced one malignant tumor on a mouse. This tumor arose at the painting site during the 17th month when 22 mice were alive in this series. It grew rapidly and soon had an ulcerated center and raised pearly edges. Histologically the tumor was an anaplastic squamous cell carcinoma, with many mitotic figures. It did not metastasize. The remaining mice show no tumors.

DISCUSSION

Tumors of rabbits' ears produced by tarring.—Papillomas and carcinomatoid tumors, but no proved carcinomas have been produced in rabbits by painting the destructive distillates of tobacco and pipe tars. The papillomas are essentially the same as the folliculo-epitheliomas of Yamagiwa and Ichikawa (57), and the common papillomas of Rous and Kidd (44).

The significance of the production of these benign papillomas in rabbits, both in these experiments and the experiments of others, is difficult to evaluate. In general these papillomatous growths are produced by known carcinogenic agents before the true carcinomas appear, but these benign tumors have not been proved to be any indication of the carcinogenic activity of the substance producing them.

In these experiments 17 carcinomatoid tumors have been seen in 11 rabbits. These tumors were definitely locally invasive, infiltrating as threads of epithelial cells both into the connective tissue and into the local lymphatic vessels. Epithelial pearls were seen occasionally. Growth through the cartilage plate and under intact epithelium occurred in one tumor. Their morphology was similar to that of a well differentiated squamous cell carcinoma. No distant metastases occurred, although in one instance what seemed to be a tumor embolus reached a subauricular lymph node. Similar tumors have been observed by others, but conclusions as to their character have varied.

Rous and Kidd (44) noted that the local invasive qualities of carcinomatoids induced by coal tar in rabbits were dependent on the continuation of tarring. These tumors did not have the unrestricted growth properties of a malignant neoplasm, and did not metastasize to distant sites. Despite long continued tarring these tumors eventually took on gross forms of old papillomas, or retrogressed. No carcinoid tumors became carcinomas.

In 1932 Roffo (32, 33) reported the production of one carcinoma with distant metastases. His subsequent papers describe and illustrate lesions produced by tobacco tars which he interpreted as carcinomas, none of which metastasized to distant sites. Obviously some of these were anaplastic squamous cell carcinomas invading and destroying the ear. However other tumors which he called carcinomas seem to be of the type called carcinomatoids by Rous and Kidd. As far as the production of papillomas and locally invasive carcinomatoid tumors are concerned, both by the application of the destructive distillate tars and the pipe tar, this study entirely confirms the work of Roffo. The main difference seems to be a matter of interpretation.

It is impossible to re-examine the earlier specimens of tar cancers in rabbits, such as those observed in the epoch-making study of Yamagiwa and Ichikawa (57). These workers obtained only a very few carcinomas which metastasized, and Rous and Kidd were convinced that many tumors which Yamagiwa and Ichikawa called carcinomas were probably carcinomatoids. Evidently it is difficult to induce cancers in rabbits by tarring which fulfill all of the criteria of malignancy.

Even with as potent a carcinogenic agent as benzpyrene the rate of carcinoma production in rabbits is low (6, 46, 49), and few metastases from what have been called carcinomas have been reported. However it is important to note that when a very active carcinogen is painted on wild cottontail rabbits' ears true squamous cell carcinomas are produced, which give rise to many distant metastases, as shown by Syverton and Berry (53), who used methylcholanthrene. The production of carcinomas of the skin of a wild rabbit's ear does not seem to be difficult with methylcholanthrene.

What then is the status of these carcinomatoid tumors? In this work with tobacco tars evidence does not indicate what the ultimate fate of these tumors would have been. Most of the tumors were identified only at autopsy, although some were seen very early in the painting period. It is of importance to note the absence of definite metastases in all 11 animals with carcinomatoid tumors. It was not proved that such tumors could produce distant metastases. In view of the work of Rous and Kidd it would seem likely that the invasive behavior of these tumors depended on the repeated application of an extrinsic stimulus rather than on an intrinsic capacity for unrestricted growth. There is no evidence that the production of these carcinomatoid tumors in rabbits is an indication of carcinogenic activity of the tobacco tars.

Carcinogenic activity of tobacco tars in mice.—Although the stock mice used in these experiments readily developed squamous cell carcinomas of the skin when painted with coal tar of known carcinogenicity, only 2 squamous cell carcinomas developed as the result of painting animals of the same stock with tobacco tars. Both tumors arose at the sites of painting. One developed after painting with the denicotinized 350-700° C. destructive distillate tar for 8.5 months, the other after 17 months' application of denicotinized pipe tar. Since no similar spontaneous tumors had been seen in some 2,000 of these mice, the production of these squamous cell carcinomas indicated that both tars had a low but definite carcinogenic activity. The earlier time of cancerization, as well as the greater capacity to produce papillomas, indicated that the activity of the 350-700° C. destructive distillate was greater than that of pipe tar. Both tobacco tars were a great deal less active than the potent coal tar used in the control experiments.

These observations are in accordance with those of Cooper, Lamb, and Sanders (11), Campbell (7, 8), and Sugiura (52), but the work of Taki (20) indicated a higher carcinogenic activity of pipe tar on mice.

Summary and Conclusions

Twelve rabbits were painted on the ears with a tar produced by the destructive distillation of tobacco at from 350-700° C. Between the 49th and 79th day all rabbits developed tumors at the painting site. The rabbits lived from 238 to over 660 days. Sixty-eight of the tumors examined histologically were papillomas, and 5 were carcinomatoid tumors. No carcinomas were produced. A 130-350° C. destructive distillate tar produced tumors in 16 out of 17 rabbits, but more slowly than the 350-700° C. tar. Forty of these tumors were papillomas and 5 carcinomatoid tumors.

Twenty-four rabbits were painted with tar obtained by smoking tobacco in pipes. Tumors were produced in from 37 to 374 days in 22 out of the 24 rabbits. Seven animals have lived over 640 days. Thirty-six tumors examined histologically were papillomas and 2 were carcinomatoid tumors. No carcinomas were produced.

These tars produced tumors less readily when painted on mice, but the 350-700° C. tar (denicotinized) produced 8 papillomas and 1 squamous cell carcinoma which arose at 8.5 months when 14 mice were alive. The pipe tar (denicotinized) produced 2 papillomas and 1 squamous cell carcinoma, which arose at 17 months when 22 mice in this series were alive.

The significance of these results is discussed.

The author wishes to acknowledge his indebtedness to Dr. H. Gideon Wells, Dr. Paul E. Steiner, and Dr. Eleanor M. Humphreys for their guidance, criticism, and encouragement of this work, and to Dr. Carl Marburg for designing the electrically heated steel still.

REFERENCES

1. Abbe, R. Cancer of the Mouth; The Case Against Tobacco. New York State J. Med., **102**:1-5. 1915.
2. Bloodgood, J. C. Cancer of the Tongue; A Preventable Disease. J. A. M. A., **77**:1381-1387. 1921.
3. Bogen, E., and R. N. Loomis. Tobacco Tar; Experimental Investigation of its Alleged Carcinogenic Action. Am. J. Cancer, **16**:1515-1521. 1932.
4. Broders, A. C. Squamous-Cell Epithelioma of the Lip. J. A. M. A., **74**:656-664. 1920.
5. Brosch, A. Theoretische und experimentelle Untersuchungen zur Pathogenesis und Histogenesis der Malignen Geschwülste. Virchows Arch. f. Path. Anat., **162**:32-85. 1900.
6. Brunschwig, A., D. Tschetter, and A. Hamann. Observations on Injections of Human Wart (Verruca Vulgaris) Extracts into Rabbits with Benzpyrene Papillomas on the Ear. Am. J. Cancer, **38**:50-54. 1940.
7. Campbell, J. A. Effects of Exhaust Gases From Internal Combustion Engines and of Tobacco Smoke upon Mice, with Special Reference to Incidence of Tumors of the Lung. Brit. J. Exper. Path., **17**: 146-158. 1936.
8. Campbell, J. A. Carcinogenic Agents Present in the Atmosphere and Incidence of Primary Lung Tumors in Mice. Brit. J. Exper. Path., **20**:122-132. 1939.
9. Chikamatsu, T. Kunstliche Erzeugung der Krebes durch Tabakteer bei Kaninchen und Maus. Tr. Jap. Path. Soc., **21**:244. 1931.
10. Cook, J. W., G. A. Haslewood, C. L. Hewett, I. Hieger, E. L. Kennaway, and W. V. Mayneord. Chemical Compounds as Carcinogenic Agents. Am. J. Cancer, **29**:219-259. 1937.
11. Cooper, E. A., F. W. M. Lamb, E. Sanders, and E. L. Hirst. Role of Tobacco Smoking in Production of Cancer. J. Hyg., **32**:295-300. 1932.
12. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 4th Ed. pp. 527, 879, 913. 1940.
13. Helwig, F. C. Growth-Producing Effects of Extracts of Tobacco on Mice. J. A. M. A., **91**:150-151. 1928.
14. Hieger, I. Fluorescence Spectrum of 3:4-Benzpyrene. Am. J. Cancer, **29**:705-714. 1937.
15. Hoffman, E., H. T. Schreus, and E. Zurhelle. Experimental Tar Tumors. Deutsche med. Wchnshr., **49**:633-624. 1923.
16. Hoffman, F. L. Cancer and Smoking Habits. Ann. Surg., **93**:50-67. 1931.
17. Hoffman, F. L. Cancer and Diet. Williams and Wilkins Co., Baltimore, 1937.
18. Kennaway, E. L. Experiments on Cancer-Producing Substances. Brit. M. J., **2**:1-4. 1925.
19. Kennaway, E. L., and B. Sampson. Tumors of Skin and Mammary Gland Caused by Pyrogenous Products of Cholesterol. J. Path. & Bact., **31**:609-612. 1928.
20. Kinoshita, R. Studies on Carcinogenic Chemical Substances. Tr. Jap. Path. Soc., **27**:665-725. 1937.
21. Lu-Fu-Hua. Über die Erzeugung von Krebs durch Tabakteerpinselung beim Kaninchen. Frankfurt. Ztschr. f. Path., **46**:513-522. 1934.
22. Lu-Fu-Hua. Über die Erzeugung von Krebs durch Tabakteerpinselung beim Kaninchen; über das Solitärauftreten einzelner Tumoren auf einer diffus gereizten Körperstelle. Frankfurt. Ztschr. f. Path., **47**:52-62. 1934.
23. Lickint, F. Tabak und Tabakrauch als ätiologischer Faktor des Carcinoms. Ztschr. f. Krebsforsch., **30**:349-365. 1929.
24. Lickint, F. Der Bronchialkrebs der Raucher. München. med. Wchnschr., **82**:1232-1234. 1935.
25. McNally, W. D. Tar in Cigarette Smoke and its Possible Effects. Am. J. Cancer, **16**:1502-1514. 1932.
26. Miescher, G., F. Almasy, and K. Kläui. Fluorescence Spectroscopic Determinations of 1:2-Benzpyrene. Biochem. Ztschr., **287**:189-197. 1937.
27. Moratti, A. Metaplasia malpighiana sperimentale della mucosa gastrica di cane. Tumori, **6**:101-139. 1932.
28. Müller, F. H. Tabakmissbrauch und Lungencarcinom. Ztschr. f. Krebsforsch., **49**:57-85. 1939.
29. Roffo, A. H. Leucoplasie tabáquica experimental. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **7**: 130-144. 1930.
30. Roffo, A. H. Desarrollo de un carcinoma en el conejo par el tabaco. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **7**:501-538. 1930.
31. Roffo, A. H. Durch Tabak beim Kaninchen entwickeltes Carcinom. Ztschr. f. Krebsforsch., **33**:321-332. 1931.
32. Roffo, A. H. Carcinome développé chez un lapin après trois ans, par l'effet du tabac. Neoplasmes, **11**:77-89. 1932.
33. Roffo, A. H. Carcinoma desarrollado en un conejo fumador a los tres anõs. Bol. Inst. de med. exper. para el etud. y trat. d. cáncer, **8**:545-573. 1931.
34. Roffo, A. H. El tabaco como carcerígeno. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **13**:287-333. 1936.
35. Roffo, A. H. Der Tabak als Krebserzeugendes Agens. Deutsche med. Wchschr., **63**:1267-1271. 1937.
36. Roffo, A. H. Espectrografia de los derivados obtenidos par destilación directa de los tabacos y su relacion como agentes cancerégenos. Bol. Inst. de med. exper. para. el estud. y trat. d. cáncer, **14**:311-399. 1937.
37. Roffo, A. H. El tabaco rubio como cancerígeno. Bol. Inst. de med. exper. para el etud. y trat. d. cáncer, **15**:5-18. 1937.
38. Roffo, A. H. Unidad cancerígena de los alquitranes des diversos tipos de tabacos. Bol. Inst. de med. exper. para el estud. y. trat. d. cáncer, **15**:349-406. 1938.
39. Roffo, A. H. Le gondron de tabac cancérigène; unité cancérigène des gondrons de divers types de tabac. Acta, Union internat. contre cancer, **4**:755-762. 1939.
40. Roffo, A. H. 1:2-benzopirene cancerígeno extraido del alquitrán del tabaco. Bol. Inst. de med. exper. para el estud. y trat. d. cancér, **16**:1-38. 1939.
41. Roffo, A. H. Krebserzeugendes Benzpyren, gewonnen aus Tabakteer. Ztschr. f. Krebsforsch., **49**:588-597. 1939.
42. Roffo, A. H. Sobre los filtros en el tabaquisino el narguité y el algodón como filtro del alquitrán de tabaco. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **16**: 255-268. 1939.
43. Roffo, A. H. Krebserzeugende Einheit der verschiedenen Tabakteere. Deutsche med. Wchnschr., **65**:963-967. 1939.
44. Rous, P., and J. G. Kidd. Comparison of Virus-Induced Rabbit Tumors with Tumors of Unknown Cause Elicited by Tarring. J. Exper. Med., **69**:399-424. 1939.
45. Rous, P., and J. G. Kidd. Activity, Transforming, and Carcinogenic Effects of Rabbit Papilloma Virus (Shope) upon Implanted Tar Tumors. J. Exper. Med., **71**:787-812. 1940.
46. Schürch, O., and A. Winterstein. Über die krebserregende Wirkung aromatischer Kohlenwasserstoff. Ztschr. f. physiol. Chem., **236**:79-91. 1935.
47. Schürch, O., and A. Winterstein. Experimentelle Untersuchungen zur Frage Tabak und Krebs. Ztschr. f. Krebsforsch., **42**:76-92. 1935.

48. Schürch, O., and A. Winterstein. Experimenteller Beitrag zur Frage Tabak und Krebs. Ztschr. f. Krebsforsch., **46**:414-419. 1937.

49. Schürch, O. Zur krebserzeugenden Wirkung des Benzpyren beim Kaninchen. Ztschr. f. Krebsforsch., **49**:353-362. 1939.

50. Shope, R. E., and E. W. Hurst. Infectious Papillomatosis of Rabbits with Note on Histopathology. J. Exper. Med., **58**:607-624. 1933.

51. Simonds, E. J. Primary Carcinoma of the Lung. Year Book Publishers Inc., Chicago, 1937.

52. Sugiura, K. Observations on Animals Painted with Tobacco Tar. Am. J. Cancer, **48**:41-49. 1940.

53. Syverton, J. T., and G. P. Berry. Sarcomata and Carcinomata Induced in Cottontail Rabbits by Methylcholanthrene. Science, **91**:298. 1940.

54. Wacker, L., and A. Schminche. Experimentelle Untersuchungen zur kausalen Genese atypischer Epithelwucherungen. München. med. Wchnschr., **58**:1607-1613. 1911.

55. Wacker, L., and A. Schminche. Experimentelle Untersuchungen zur kausalen Genese atypischer Epithelwucherungen. München. med. Wchnschr., **58**:1680-1682. 1911.

56. Watson, A. F. Experimental Skin Tumors in Rat Produced by Tar. J. Path. & Bact., **36**:251-256. 1933.

57. Yamagiwa, K., and K. Ichikawa. Experimental Study of the Pathogenesis of Carcinoma. J. Cancer Research, **3**:1-21. 1918.

The Relation of Age to the Occurrence of Adenoma-Like Lesions in the Rat Hypophysis and to Their Growth After Transplantation*

John A. Saxton, Jr., M.D.

(From the Department of Pathology, Cornell University Medical College, New York, N. Y.)

(Received for publication March 14, 1941)

The spontaneous occurrence of chromophobe adenomas and focal hyperplasia in the rat hypophysis has been reported by Wolfe, Bryan, and Wright (18), and by Oberling, Sannié, and the Guérins (13). Hyperplasia of chromophobic cells and the development of chromophobe adenomas in the rat hypophysis following injection of estrogens have been described by Wolfe and Wright (19), by Zondek (20), and by McEuen, Selye, and Collip (11). References to tumors of the rat hypophysis, either spontaneous or induced, are not frequent in the literature, and no reference to attempts at transplantation could be found.

The purposes of this paper are to report the high incidence with advancing age of adenoma-like lesions of chromophobe cells in a strain of albino rats, to describe an experiment in which successful growth was obtained by homologous transplantation, and to discuss the possible factors necessary for growth following transplantation. The results of assays of adenoma-like tissue for hypophyseal hormones are also described.

Materials and Methods

A total of 107 male and 20 female albino rats, ranging in age from 1 month to 30 months, were examined during the course of this study. These animals were derived from the Yale strain and represented normal stock rats from the colony maintained by the Department of Animal Nutrition at Cornell University. The pathological findings in other organs of this strain of rat maintained upon various experimental regimens are described elsewhere by McCay and others (10), and by Saxton and Kimball (16). Nodular hyperplasia of the hypophysis has been noted occasionally during routine autopsies on animals of this colony, but the incidence has not been tabulated for the entire group. The animals described here may be considered an adequate cross section of the colony with respect to age.

In each case the hypophysis was examined grossly and microscopic sections were prepared from glands showing gross evidence of abnormality. When the high incidence of these lesions became apparent toward the end of the study, routine sections were prepared from all hypophyses examined.

The terms nodular hyperplasia and adenoma-like lesions of chromophobe cells are used in this report because it is often impossible to differentiate on morphological grounds between a nodule of hyperplastic tissue and a true adenoma.

Incidence and Morphology

In Table I is given the observed incidence of nodular hyperplasia of chromophobe cells in relation to age. Since microscopic examination of hypophyses was made largely in cases suspected of containing lesions, the true incidence in older animals is probably greater than indicated. No lesion was observed in 84 animals up to 13 months of age. The youngest rat to bear either a gross or a microscopic nodule was 15 months of age. The incidence in rats of 13 months of age and over was 39 per cent, lesions of this sort having been found in 17 of 43 rats of this age group. Most of the animals examined were males, and 16 of the 17 lesions were found in this sex. One typical adenoma-like growth was found in a female. The hypophysis in this case weighed 37.5 mgm.

Table I: Incidence of Chromophobe Adenoma-Like Lesions in Relation to Age

Age (months)	Number of rats examined (sex)	Number of rats with hypophyseal lesions (sex)	Incidence (per cent)
1-12	84 (77 M, 7 F)	0	0
13-30	43 (30 M, 13 F)	17 (16 M, 1 F)	39

The gross appearances varied from small yellow nodules 1 mm. in diameter seen on the under surface of the hypophysis or in its lateral part, to a large nodular mass of soft pinkish yellow tissue measuring 5 mm. in its greatest diameter and compressing the posterior lobe and adjacent normal areas of hypophysis. The weights of hypophyses bearing nodules of hyperplasia ranged from 14.7 mgm., where only a single microscopic nodule was found, to 159 mgm. In one

*This investigation was aided by a grant from the Josiah Macy, Jr., Foundation.

hypophysis which weighed 16.7 mgm., a symmetrical enlargement of the lateral parts was present, and in section two apparently unjoined adenoma-like masses were found, one on either side of the midline. Asymmetrical enlargement was more common.

Microscopically, all of the lesions observed were similar in most respects and resembled either the nodular foci of chromophobes or chromophobe adenomas described by Wolfe, Bryan, and Wright (18). However, one lesion resembled the hemorrhagic or cavernous type of growth described in the experiments cited (11, 19). The nodules with few exceptions were composed of groups or cords of slightly enlarged chromophobes associated with abundant vascular channels. The nuclei were usually large. The cytoplasm was usually pale and agranular, and contained numerous fat vacuoles of various sizes. When stained with Sudan III, the nodules stood out in sharp contrast to the normal hypophyseal tissue, in which no fat was demonstrable. The adenoma-like areas were not encapsulated or sharply delimited from the adjacent normal tissue, but in every case examined the adjacent tissue was compressed in some degree. Invasion of adjacent structures was never seen, and in all glands bearing lesions some normal hypophyseal tissue was present. In the majority of cases the growths seemed to have multicentric origins. Usually a nodule was found in each lateral part of the hypophysis, but not always in the same relative position. In several hypophyses three or more apparently separate nodules were observed (Fig. 1). Mitotic figures were often abundant even in the smallest lesions. Differences in size of nodules within the same pituitary suggested variations either in time of origin or in rate of growth. In the largest adenoma-like growth, which was used for assay and transfer, the chromophobe cells assumed a character not seen in the other cases. At the borders of the lesion a regular arrangement of vacuolated chromophobes was seen; but near the center were numerous large cells with pale cytoplasm and hyperchromatic nuclei. These nuclei assumed bizarre shapes and multinucleated cells were not uncommon. Mitotic figures were present in this area, as well as in the peripheral regions. No necrosis or hemorrhage was found in a section through the longest diameter of the lesion (Fig. 2).

No consistent change was found in other endocrine organs of rats bearing these hypophyseal lesions, but in several instances the prostate and seminal vesicles were enlarged.

Assay of Adenoma-Like Tissue for Hypophyseal Hormones

Adenoma-like tissue from the largest hypophysis encountered was subjected to assay for hypophyseal hormones and also was used for transplantation. The hypophysis bearing this lesion weighed 159 mgm. and was found in a rat 17 months old which had been killed with chloroform. Microscopic sections of this gland showed that the normal hypophyseal tissue had been pushed far to one side by the growth of chromophobe cells. The tissue used for assay and for transfer was taken from the central portion of the growth. No normal hypophyseal structure was recognized microscopically in sections contiguous with that selected. It seemed reasonably certain, therefore, that normal hypophyseal tissue was excluded both from the tissue used for assay and from the tissue used for transfer.

The method of assay is described in detail elsewhere (15). Two immature female guinea pigs were used as test animals. One received a total of 40 mgm. of fresh adenoma-like tissue in divided doses subcutaneously daily for 4 days, and was examined on the succeeding day. The thyroid, ovaries, and adrenal glands were examined grossly and by microscopic section. The same procedure was carried out with the second test animal, using 20 mgm. of tissue. Control assays were carried out using comparable amounts of normal hypophyseal tissue from rats of the same age and strain.

The ovaries, thyroids, and adrenals of the 2 guinea pigs receiving 40 and 20 mgm. of adenoma-like tissue respectively, showed no evidence of stimulation. Similar amounts of hypophyseal tissue from normal rats produced in the controls a moderate hypertrophy of the thyroid, follicular growth and maturation, as well as conspicuous luteinization in the ovaries, and

DESCRIPTION OF FIGURES 1 TO 5

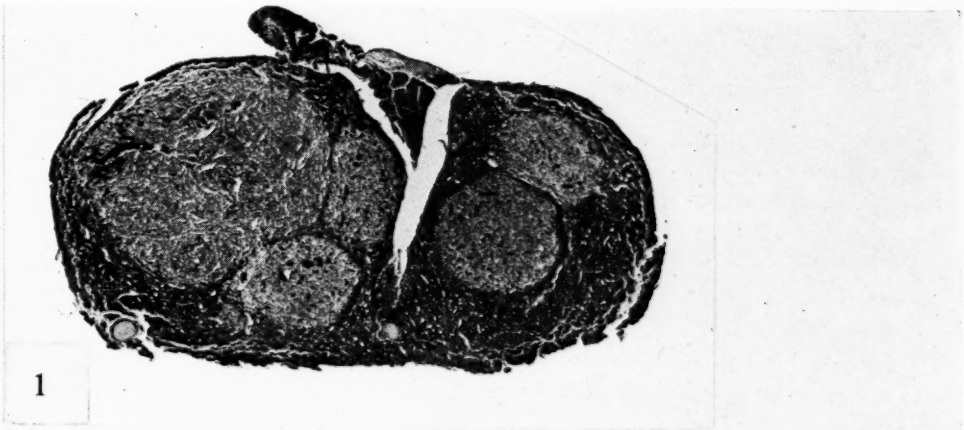
Fig. 1.—Cross section of hypophysis of male rat 15 months old, showing 5 separate adenoma-like nodules of chromophobe cells. × 17.

Fig. 2.—Representative area of chromophobe adenoma-like lesion used for transplantation and bioassay, showing variation in size of cells, moderate vacuolization of cytoplasm, and mitotic figures. × 400.

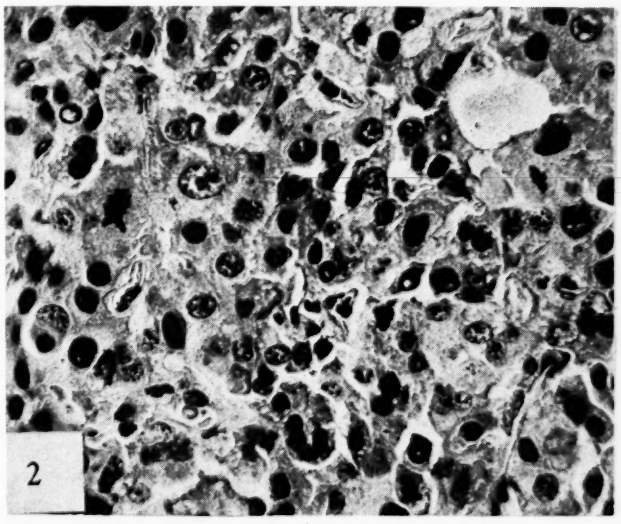
Fig. 3.—Representative area of successful transplant of adenoma-like tissue, shown in Fig. 2, removed 10 months after transplantation. Several mitotic figures are present and the cytoplasm of many cells is vacuolated. × 400.

Fig. 4.—Photograph of rat eye containing transplant of adenoma-like lesion shown in Fig. 2. The photograph was taken 15 months after transplantation. The transplant is seen as a rounded mass in the anterior chamber nearest the posterior palpebral commissure. × 3.

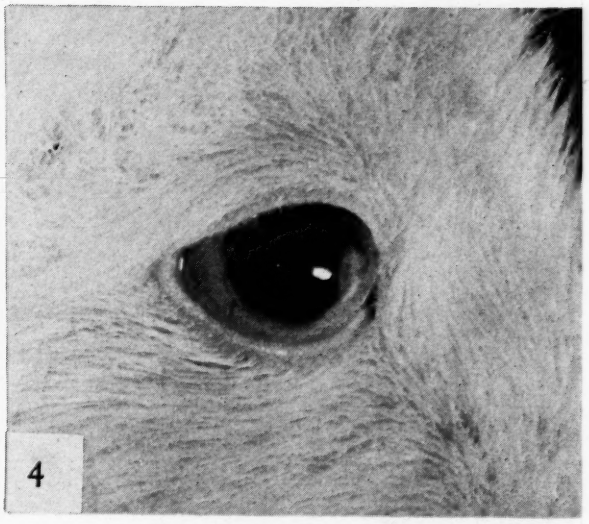
Fig. 5.—Same eye as shown in Fig. 4, photographed 19 months after transplantation. The transplant has approximately doubled in size during the 4-month period between photographs. × 3.



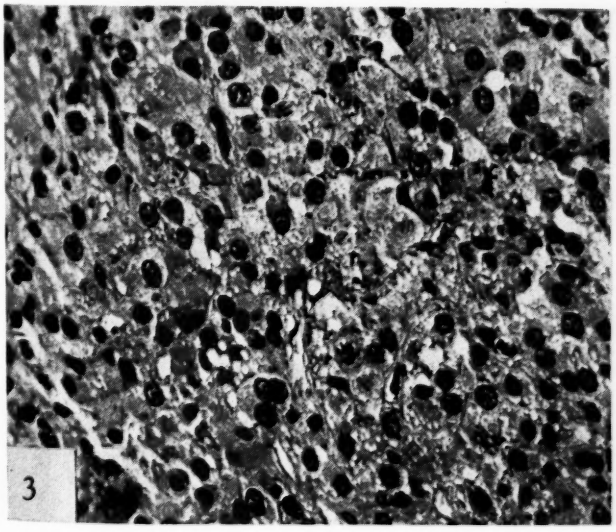
1



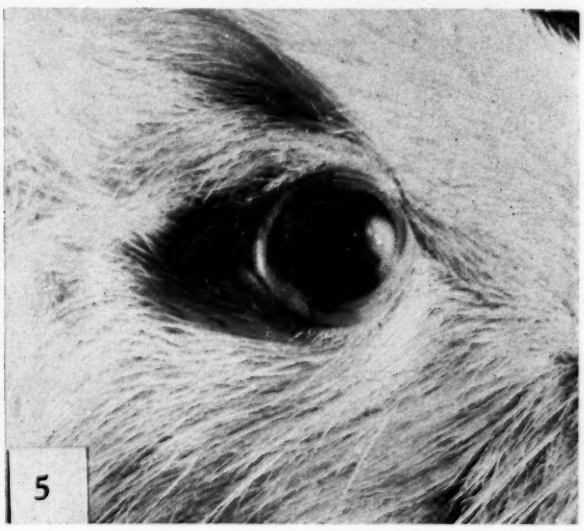
2



4



3



5

FIGS. 1-5

enlargement of the adrenals with an increase in mitotic figures in the cortex.

Transplantation

Small pieces of the largest adenoma-like lesion were transplanted into the anterior chamber of one eye of each of 9 male rats of the same strain, 2 to 3 months of age. In each instance the tissue was visible for a short period, but in 6 of the rats it had disappeared completely after 5½ months had elapsed. The remaining 3 rats were observed at short intervals over the course of more than a year and the observations in each of these 3 cases are described separately below.

Rat No. 1.—The transplant remained visible as a small pinkish-yellow fleck for several months, gradually assuming a rounded shape, but not perceptibly increasing in size. From the color it was considered that a vascular supply had become established. After 7 months the tissue was noticeably enlarging. The mass became bilobate, with rounded margins, and at the end of 10 months occupied about one quarter of the anterior chamber. At this time the eye was enucleated and the tissue used for section and second serial transfer. Section revealed a well vascularized nodular growth of chromophobe cells similar to those of the original lesion. A thin capsule of fibrous tissue surrounded the transplant. Cytoplasmic vacuoles were present, and nuclei were relatively large; but no bizarre forms were seen. The entire transplant appeared viable, and contained many mitotic figures (Fig. 3).

From this transplant a second series of transfers was made into 6 young rats. In only 2 of these did the tissue persist, and these rats are at present under observation. After 8 months each transplant shows slight evidence of growth.

The rat from which the eye with its transplant was removed was killed, 4 months later, when it had reached the age of 17 months. The pituitary was grossly normal. On section several small foci of chromophobe cells with vacuolated cytoplasm were found. There was no adenoma-like lesion. A mitotic figure was seen in one of the foci.

Rat No. 2.—Growth of the transplant was not evident until 10 months after inoculation. During the ensuing 3 months a relatively rapid growth took place. In gross appearance the transplant was identical with that already described. This rat was found dead at the age of 16 months, or 3 months after growth was first noted. The transplant in the eye was a well rounded, orange-yellow mass, weighing 9.7 mgm. and measuring 4 by 3 mm., as seen through the cornea. Microscopic section resembled in all respects the transplant previously described, except that an occasional large hyperchromatic nucleus was seen. There was no necrosis in the transplant. The pituitary of this rat weighed 14.7 mgm. and was of normal gross appearance. On section, however, it contained a small, sharply outlined nodule of hyperplasia of chromophobe cells, measuring 1.5 by 1 mm. The cytoplasm of many of the cells contained vacuoles. This nodule is included in the tabulated incidence of adenoma-like lesions.

Rat No. 3.—Slight enlargement of the transplant was noted after about 12 months. After 15 months it appeared as a rounded, orange-yellow mass 2 mm. in diameter, as seen through the cornea (Fig. 4). Between the 15th and 19th months after transfer it had approximately doubled in size (Fig. 5). The rat bearing this transplant was killed at 2 years of age, or 21 months after transplantation. The transplant was a yellow lobate nodule measuring 3.5 by 2.5 by 2 mm. It had a thin capsule and was easily shelled out of the anterior chamber. The tissue was used for section and for a second serial transfer. Section revealed a well vascularized nodule composed of chromophobe cells similar to the original lesion. In a few of them the cytoplasm contained vacuoles. An occasional mitotic figure was found. From this animal second serial transfers were made into the anterior chambers of 4 young and 4 old male rats and these rats are at present under observation.

The hypophysis of rat No. 3 was greatly enlarged and the anterior and posterior lobes appeared to be pushed forward by a relatively large hyperemic mass measuring 5 by 4 by 3 mm. and continuous with the anterior lobe. A considerable amount of fresh blood escaped during removal and the lesion then appeared sponge-like in texture. After washing in saline the weight of the hypophysis bearing this lesion was 25 mgm.

Microscopically, the normal anterior lobe tissue was slightly compressed at the margin of the nodule that has been described. This lesion had no capsule, but was sharply demarcated from the normal tissue by the character of the cells. It consisted of an adenoma-like growth of moderately enlarged chromophobe cells, which were arranged in cords and trabeculations of varying thickness. Small blood vessels were seen within the larger of the cords. Between the cords were large spaces which before removal of the hypophysis appeared to be filled with blood and now contained a few red cells. They had no endothelial lining. In one place in the stroma were several phagocytes laden with brown pigment. The lesion appears to be identical with the hemorrhagic or cavernous adenomatous growths described by several observers (11, 19).

Discussion

The high incidence of chromophobe hyperplasia or adenoma-like lesions in older rats suggests that ageing

processes may play a part in their development. Wolfe and Wright (19), Zondek (20), and McEuen, Selye, and Collip (11) have shown that continued administration of estrogens favors development of these tumors, and the former authors (19) have suggested that an endocrine imbalance in ageing rats may induce hyperplasia of the chromophobe elements. The multicentric origin of the lesions favors this view. The high incidence in the strain here described and in the strains used in the experiments cited (13, 18) suggests that this is a relatively common lesion of old rats. It may occur in either sex. Parsons (14) and Costello (1) have independently shown that small adenoma-like lesions of various types and foci of cellular hyperplasia are frequent in the hypophysis of man, and increase in number with age.

The adenoma-like tissue that was assayed did not contain detectable amounts of gonadotropic, thyrotropic, or adrenal cortex stimulating hormones. It is usually believed that diminution of hormones in the hypophysis indicates diminution or even absence of production of hypophyseal hormones. The results of the assay are in agreement with the generally accepted opinion that chromophobe tumors in man do not produce clinical evidence of secretory function. Zondek (20), however, assayed adenomatous pituitaries of rats and found a normal level of gonadotropic hormones. The entire hypophysis was assayed, whereas in these experiments only adenoma-like tissue was used. The results of the control assays of normal hypophyses are in accord with the findings of Loeb (6).

It is unfortunate that little fresh material was available for transplantation. Although the numbers of transplants are too small to warrant conclusions, certain inferences may perhaps be drawn from the behavior of the three successful transplants. In each instance there has been a long latent period followed by slow growth. Growth of the transplants was not noted until periods of 7 to 12 months had elapsed, and when growth did occur, the rate was considerably slower than in the case of malignant tumors transplanted to the anterior chamber (4). A slow and irregular rate of growth following transplantation of benign tumors has been found with transplanted adenofibromas of the rat, by Loeb and Fleisher (7) and by Heiman (5). However, in the experiment cited, the transplants grew more rapidly and attained a size greater than that of the transplants of hypophyseal adenoma-like tissue. The small volume of the rat's eye does not seem to have been a limiting factor for growth, because in no instance was more than one third of the anterior chamber occupied by the transplant. It seems probable that the hypophyseal lesion here studied is more benign in character than the adenofibromas described by Loeb and Fleisher, and that it has a lower growth energy.

The significance of the prolonged latent period, which was from 7 to 12 months, was not evident. It is possible that a long latent period following transplantation is merely an expression of the benign character of these growths. In the experiments of Loeb and Fleisher, the latent periods following the initial transfer of benign tumors ranged up to 4 months, and in Heiman's experiments there was wide fluctuation of growth energy. On the other hand, it is possible that advanced age of the host may be necessary for growth of this type of tissue following transplantation, whereas on the contrary, according to the usual experience, young animals are more suitable for the transplantation of tumors (8). In the period of several months during which the transplants persisted but did not grow, the hosts were approaching the period of life in which these nodules might develop spontaneously. It is conceivable that internal factors responsible for the spontaneous hyperplasia may then induce proliferation of cells in the transplant. It is noteworthy that two of the hosts developed in their own hypophyses similar adenoma-like growths. This is perhaps analogous in certain respects to the observation of Fleisher and Loeb (2) and of Greene (3), showing that transplantation of mammary tumors was more frequently successful in hosts with spontaneous growths.

If this interpretation is correct, the adenoma-like lesion has scant, if any, autonomy of growth, but is dependent upon a specific stimulus provided by the host. Growth may be compared to that which occurs in a specialized tissue introduced into a medium where certain stimuli act upon it. In this respect the growth resembles the physiological responses obtained in normal uterine and prostatic tissue transplanted into the anterior chamber (9, 12, 17). The static condition of the second serial transfer indicates that increased energy of growth was not developed by transplantation. Further experiments are necessary to test the validity of these inferences.

From a consideration of the morphology and observed behavior of these lesions it is difficult to draw a sharp line between focal hyperplasia and truly adenomatous proliferation. In the largest nodule encountered there were areas closely resembling neoplastic growth. Such areas were not observed in the smaller and presumably younger tumors, although mitotic figures were frequent. If original growth is dependent upon stimulating factors from the host, then a stronger stimulus or perhaps a longer period of stimulation may bring about changes more closely identified with those of neoplasms. The largest tumor described has the morphological characters of a chromophobe adenoma, even though the behavior of the transplants indicated little if any autonomy of growth. Whether all of the smaller growths observed

represent adenomas in a strict sense is open to question. These observations suggest a close relationship between focal hyperplasia and growth of an adenomatous nature, and it is doubtful if this characteristic lesion of the hypophysis is analogous to adenomas of other organs. Adenoma-like hyperplasia is perhaps a more accurate designation for these lesions.

It seems unlikely that the lipoid content of the cells in the nodules is evidence of cellular degeneration. Nuclear degeneration was not observed and fat-laden cells were present in areas of active proliferation. A high lipoid content may be considered as a characteristic of the chromophobe cells comprising these lesions, being absent in only one; but its significance is not clear.

Summary

Chromophobe adenoma-like lesions of the hypophysis occur frequently at an advanced age in albino rats of the Yale strain. Adenoma-like lesions were found in the hypophyses of 17 of 43 rats aged between 1 and 2 years, whereas none was found in 84 rats of less than 1 year of age. Conspicuous morphological features were frequent multicentric origin of the nodules and abundant lipoid vacuoles in the chromophobe cells.

Bioassay of adenoma-like tissue failed to indicate the presence of gonadotropic, thyrotropic, or adrenotropic hormones. These hormones were demonstrated in comparable amounts of normal rat hypophyseal tissue.

Growth of homologous intra-ocular transplants of adenoma-like tissue occurred in three out of nine instances after latent periods of 7, 10, and 12 months, at a time when the recipients had reached an advanced age.

The advanced age at which adenoma-like lesions appear in the hypophysis, their multicentric origin, and the prolonged latent periods of the transplants suggest that they are dependent upon factors in the host which make their appearance at an advanced age. They have perhaps no autonomous growth and represent hyperplasia in response to conditions that appear with advancing age.

The animals used in this study were made available through the kindness of Dr. C. M. McCay.

REFERENCES

1. Costello, R. T. Subclinical Adenoma of the Pituitary Gland. Am. J. Path., **12**:205-215. 1936.
2. Fleisher, M. S., and L. Loeb. Transplantation of Tumors in Animals with Spontaneously Developed Tumors. Surg., Gynec. & Obst., **17**:203-206. 1913.
3. Greene, H. S. N. Familial Mammary Tumors in the Rabbit. IV. The Evolution of Autonomy in the Course of Development as Indicated by Transplantation Experiments. J. Exper. Med., **71**:305-324. 1940.
4. Greene, H. S. N., and J. A. Saxton, Jr. Uterine Adenomata in the Rabbit. I. Clinical History, Pathology and Preliminary Transplantation Experiments. J. Exper. Med., **67**:691-708. 1938.
5. Heiman, J. The Study of Benign Neoplasms of the Rat's Breast. Am. J. Cancer, **22**:497-524. 1934.
6. Loeb, L. Effects of Anterior Pituitary from Various Species on Sex and Thyroid of Immature Guinea Pigs. Proc. Soc. Exper. Biol. & Med., **29**:1128-1131. 1932.
7. Loeb, L., and M. S. Fleisher. Transplantation of Benign Tumors. J. Cancer Research, **1**:427-459. 1916.
8. MacCallum, W. G. Textbook of Pathology. W. B. Saunders Co., Philadelphia, 7th Ed. 1940.
9. Markee, J. E. Rhythmic Variations in the Vascularity of the Uterus of the Guinea Pig During the Estrous Cycle. Am. J. Obst. & Gynec., **17**:205-208. 1929.
10. McCay, C. M., G. H. Ellis, LeR. L. Barnes, C. A. H. Smith, and G. Sperling. Chemical and Pathological Changes in Ageing and After Retarded Growth. J. Nutrition, **18**:15-25. 1939.
11. McEuen, C. S., H. Selye, and J. B. Collip. Some Effects of Prolonged Administration of Oestrin in Rats. Lancet, **1**:775-776. 1936.
12. Moore, R. A., H. B. Rosenblum, S. H. Tolins, and R. H. Melchionna. Variation in the Size of Transplants of the Prostate and Seminal Vesicle in the Anterior Chamber of the Eye. J. Exper. Med., **66**:273-279. 1937.
13. Oberling, C., C. Sannié, P. Guérin, and M. Guérin. Sur la relation apparente des tumeurs hypophysaires et du benzpyrène injecté dans le cerveau chez le rat. Compt. rend. Soc. de biol., **131**:455-457. 1939.
14. Parsons, R. J. The Pituitary Gland and its Relation to Age, Hypertension and Pathological Processes; A Study of 107 Unselected Pituitaries. Med. Papers Dedicated to Dr. Henry A. Christian, 366-405. 1936.
15. Saxton, J. A., Jr., and H. S. N. Greene. Age and Sex Differences in Hormone Content of the Rabbit Hypophysis. Endocrinology, **24**:494-502. 1939.
16. Saxton, J. A., Jr., and G. C. Kimball. To be published.
17. Schochet, S. S. Experimental Endometriosis. Am. J. Obst. & Gynec., **17**:328-344. 1929.
18. Wolfe, J. M., W. R. Bryan, and A. W. Wright. Histologic Observations on the Anterior Pituitaries of Old Rats with Particular Reference to the Spontaneous Appearance of Pituitary Adenomata. Am. J. Cancer, **34**:352-372. 1938.
19. Wolfe, J. M., and A. W. Wright. Histologic Effects Induced in the Anterior Pituitary of the Rat by Prolonged Injection of Estrin with Particular Reference to the Production of Pituitary Adenomata. Endocrinology, **23**: 200-210. 1938.
20. Zondek, B. Hypophyseal Tumors Induced by Estrogenic Hormone. Am. J. Cancer, **33**:555-559. 1938.

Adrenal Glands of Mice from Strains with Different Susceptibilities to Mammary Carcinoma*†

J. S. Blaisdell, M.D., W. U. Gardner, Ph.D., and L. C. Strong, Ph.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

(Received for publication March 3, 1941)

A number of recent investigations have been interpreted to indicate that degenerative lesions of the adrenal glands are associated with the development of mammary carcinoma in inbred mice of tumor-susceptible strains (4-7). According to this hypothesis the adrenal cortex or its hormones acts in some way to fortify the action of estrogens on the mammary glands, while the adrenal medulla opposes this action. Therefore, the location, in addition to the occurrence of an adrenal degenerative process, which was designated "brown degeneration" is supposed to be related etiologically to the development of mammary carcinoma. According to Cramer (5), brown degeneration involving the adrenal medulla of female mice of tumor-susceptible strains reduces the antagonistic action of the medulla to the mammary stimulating effect of estrogen. Therefore, estrogen uninhibited by the degenerated medulla is free to act upon the mammae to initiate cancerous growth.

Cramer (4) interpreted brown degeneration to be a "vague manifestation of susceptibility to the development of cancer," and regarded the presence of this process as a "visible manifestation of an adequate mammary stimulus to breast cancer development." Kreyberg and Ecker (11) were unable to support this interpretation after investigating the problem in several lines of inbred mice with variable susceptibilities to occurrence of tumor. Brown degeneration was considered an inherent or genetically determined process distinct from intrinsic susceptibility to cancer.

The typical appearance and morphology of brown degeneration has been described by several investigators (9, 10). Its significance, if any, has been the subject of much conjecture and many interpretations (9, 10, 12, 14). Because of the evident lack of agreement as to its significance, and because of the availability of 11 inbred strains of mice with varying potentialities for development of mammary carcinoma, a further investigation of this problem was undertaken to ascertain the possibility of a relationship of brown degeneration or other aspects of adrenal morphology to the development of mammary carcinoma in mice.

TABLE I: THE SEX DISTRIBUTION AND THE FREQUENCY AND MEAN AGE OF DEVELOPMENT OF MAMMARY CARCINOMA IN 11 STRAINS OF MICE

Strain	Total no. of animals	No. of females	No. of males	Frequency of mammary carcinoma development	Mean age of carcinoma development, in months (Data of Strong)
C3H	33	27	6	+++	8
A	13	10	3	+++	12
JK	14	7	7	—	..
C12I	13	8	5	++	14
C57	14	12	2	—	..
CBA	13	10	3	+	24
L	10	6	4	—	..
N	8	7	1	—	..
CHI	6	6	0	++	20
NH	3	3	0	—	..
F	3	3	0	—	..
Total	130	99	31		

+++ = Mammary carcinomas are very common, high cancer susceptibilities.
++ = Mammary carcinomas are frequent, intermediate cancer susceptibilities.
+ = Mammary carcinomas occur sporadically, low cancer susceptibilities.
— = Mammary carcinomas rarely or never occur, cancer-resistant.

MATERIALS AND METHODS

Adrenal glands from 130 mice of 11 inbred strains [1] with varying susceptibilities for the development of mammary tumors were examined in this investigation. The number of animals from each strain, the number of each sex, the relative tumor susceptibility, and the mean age at the time of development of mammary carcinoma are recorded in Table I. The age of the animals ranged from 97 to 770 days. Eleven animals received weekly subcutaneous injections of oil of benne. The remaining animals received no treatment but

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Anna Fuller Fund.

† This paper is based upon a thesis submitted by J. S. Blaisdell to the Faculty of the School of Medicine, Yale University, June 1940, in candidacy for the degree of Doctor of Medicine.

[1] Ten of the 11 strains of mice were developed by L. C. Strong. One strain (C57) was obtained from Dr. H. S. Bagg and has been continued in this laboratory. The data on the frequency of mammary tumors were obtained from the records in the laboratory.

were used either as breeders or controls for other experiments in progress in the laboratory. The diet of the mice consisted of Fox Chow or Nurishmix, and in addition, water was always available.

The animals whose adrenal glands were studied were killed by illuminating gas and promptly autopsied. The glands were removed and usually fixed in Bouin's solution. Occasionally formalin or Helly's fixative was used. Serial sections were cut either of the whole gland or from the central portions. These sections were stained with Ehrlich's hematoxylin and eosin. Iodine or crystal violet were also employed on certain selected sections when amyloid was suspected.

A preliminary microscopic examination of all the slides was made in order to determine the range of morphological variation of the adrenals in mice and to develop a method of classification and tabulation. The glands were carefully re-examined and classified as to strain, sex, and age, and the microscopic findings were tabulated (Table II).

Brown degeneration.—For uniformity and comparison the method of Cramer (5) was used in classifying the extent of brown degeneration as follows:

0—"No evidence of any brownish-colored cells anywhere in the corticomedullary junction where they usually first appear.

1—"Enlarged brown cells in the reticularis containing lipoid material.

2—"Several of these enlarged cells fuse to form isolated brown masses. (Fig. 1.)

3—"As these masses increase in size, they coalesce and form larger masses which are still separated from each other and are mainly confined to the zona reticularis.

4—"Eventually these enlarging masses unite to form an uninterrupted brown ring situated around the medulla and gradually extend into its peripheral cells. Sometimes such an extension occurs even in the preceding stage." (Fig. 2.)

Amyloid degeneration or amyloid change.—This was observed in about one-half of the glands and was classified in stages as follows:

0—None present.

TABLE II: A SUMMARY OF THE DISTRIBUTION AND EXTENT OF BROWN AND AMYLOID DEGENERATION IN ADRENALS OF MALE AND FEMALE MICE AND THE INCIDENCE OF MAMMARY CARCINOMA IN THESE MICE

Strain	Sex	No. animals	Brown degeneration stages					Total brown degeneration	Per cent brown degeneration	Amyloid degeneration stages					Total amyloid degeneration	Per cent amyloid degeneration	Total carcinoma	Per cent carcinoma
			0	1	2	3	4			0	1	2	3	4				
C3H	F	27	4	21	2	..	..	23	85.2	16	7	1	..	..	8	29.6	22	81.5
	M	6	1	5	..	..	..	5	83.3	3	1	1	..	..	2	33.3	..	..
	F,M	33	5	26	2			28	84.8	19	8	2			10	33.3	22	..
A	F	10	8	2	..	..	..	2	20.0	3	4	3	..		7	70.0	4	40.0
	M	3	3	..	..	..	..	..	..	..	..	1	..	2	3	100.0	..	..
	F,M	13	11	2	..	..		2	15.3	3	4	4		2	10	76.9	4	..
C12I	F	8	5	2	..	1	..	3	37.5	3	4	1	..		5	62.5	2	25.0
	M	5	5	..	..	..	..	..	..	2	2	..	..	1	3	60.0	..	..
	F,M	13	10	2	..	1	..	3	23.1	5	6	1		1	8	61.5	2	..
CHI	F	6	3	3	..	..	..	3	50.0	..	4	..	2		6	100.0	2	.
CBA	F	10	1	3	6	..	..	9	90.0	10	..	..	..	..	..	..	1	..
	M	3	3	..	..	..	..	..	..	3	..	..	..	..	..	..	..	..
	F,M	13	4	3	6	..	..	9	69.2	13	..	..	..	..	..	..	1	..
Totals	F	61	21	31	8	1	..	40	65.6	32	19	5	2	..	26	42.6	29	47.5
	M	17	12	5	..	..	..	5	29.4	8	3	2	..	3	8	47.0	..	..
	F,M	78	30	36	8	1	..	45	57.7	40	22	7	2	3	34	43.8	..	..
JK	F	7	4	3	..	..	..	3	42.9	1	5	1	..	..	6	85.7	..	..
	M	7	6	..	..	..	1	1	14.3	5	1	..	..	..	1	14.3	..	..
	F,M	14	10	3	..	..	1	4	28.6	6	6	1	..	..	7	50.0	..	..
C57	F	12	..	8	4	..	..	12	100.0	10	..	..	2	..	2	16.7	..	..
	M	2	1	1	..	..	..	1	50.0	2	..	..	..	..	..	..	..	..
	F,M	14	1	9	4	..	..	13	92.9	12			2	..	2	14.3	..	..
L	F	6	3	3	..	..	..	3	50.0	3	1	..	1	1	3	50.0	..	..
	M	4	..	4	..	..	..	4	100.0	3	1	..	..	.	1	25.0	..	..
	F,M	10	3	7	..	..	..	7	70.0	6	2	..	1	1	4	40.0	..	..
N	F	7	..	..	2	2	3	7	100.0	5	2	..	..	..	2	28.6	..	..
	M	1	..	..	..	1	..	1	100.0	..	1	..	..	..	1	100.0	..	..
	F,M	8	.	.	2	3	3	8	100.0	5	3	.	..	..	3	37.5	..	..
NH	F	3	.	2		1		3	100.0	3						..	..	..
F	F	3	1	1	1	..	..	2	66.7	2	1				1	33.3	..	..
Totals	F	38	8	17	7	3	3	30	79	24	9	1	3	1	14	36.8	..	..
	M	14	7	5	..	1	1	7	50	10	3	..	..	..	3	21.4	..	..
	F,M	52	15	22	7	4	4	37	71.1	34	12	1	3	1	17	32.7	..	..

1—Small collections of amyloid in the central ends of the sinusoids of the cortex. It may be spotty in appearance or may form a narrow band about the medulla. Small, isolated collections may be present in mid-cortex.

2—The collections of amyloid may extend outward along the cortical sinusoids in a spokelike fashion or may form a thicker concentric band about the medulla. A progressive degeneration of adjacent cortical cells becomes evident at this stage and the amyloid extends out to mid-cortex.

3—The process has advanced nearly to the zona glomerulosa. Degeneration of the cortical cells is extensive. The typical architecture is disrupted, especially in the perimedullary zone, and particles of cellular debris may be seen suspended in the amyloid material. (Fig. 3.)

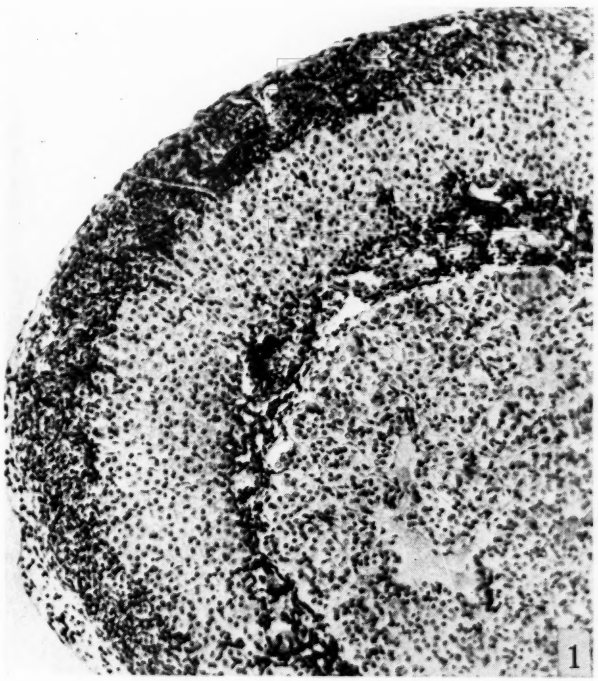


FIG. 1.—Photomicrograph of a section of an adrenal gland of a mouse showing brown degeneration (Stage 2). The large brown cells are limited to the perimedullary zone. The glomerulosa is thick and contains many round cells in addition to adrenal cells. × 90.

4—Amyloid has filled, or nearly filled, the entire cortical region and considerable encroachment upon the medulla may also be observed. The glomerulosa consists of only a "rind" of degenerating parenchymal cells. (Fig. 4.)

OBSERVATIONS

The morphology of the adrenal glands of mice and the structural nature and staining reactions of the brownish perimedullar mass termed brown degeneration have been adequately described (1, 2, 4, 6). Brown degeneration apparently first appeared as the accumulation of a pigmented material in the cortical cells in the perimedullary region. In some animals multinucleate masses, several times larger than a normal cortical cell, containing vacuoles and brownish pigment were scattered around the medulla. These large cells or masses occurred in large numbers in the adrenal glands of other mice and formed a continuous ring broken only by a few sinusoids at the inner portion of the cortex. The greater part of the zona fasciculata may be replaced by brown degeneration in other adrenals. It was assumed that these changes were progressive, that the adrenals showing the most extensive degeneration (Stage 4) previously had shown only a slight accumulation of pigmented material.

A second degenerative process of the adrenal glands

FIG. 2.—Section of the adrenal gland of a mouse showing extensive brown degeneration (Stage 4). A broad band of pigmented material has replaced the greater part of the adrenal cortex. × 90. Slightly retouched along boundary between the cortex and medulla.

of mice was classified as amyloidosis because of the positive staining reaction of this material with iodine and crystal violet, as well as its appearance in hematoxylin and eosin preparations. The relative amount of amyloid degeneration of the adrenals of different animals varied greatly as indicated previously and it was assumed that the process was probably continuous. Amyloid was first detected at one or more areas in the perimedullary region or in the deeper portion of the zona fasciculata. In some adrenals the greater part of the cortex was involved (Stage 4). Such adrenals were grossly enlarged and light red in color rather than opaque yellowish or cream-colored.

The relation of susceptibility to develop mammary

carcinoma to adrenal changes.—Of the 130 mice studied, 99 were females and 31 were males. Sixty-one of the females were from the 5 cancer-susceptible strains studied and 38 from the cancer-resistant strains. The adrenals of 65.6 per cent of the former and 79 per cent of the latter group showed brown degeneration (Table II). Of the 31 males, 17 were from the cancer-susceptible strains and 14 from the cancer-resistant strains. Brown degeneration appeared in the adrenals of 29 per cent of the former and 50 per cent of the latter group.

Amyloid degeneration was present in the adrenals of 42.6 per cent of the cancer-susceptible and of 36.8 per cent of the cancer-resistant females (Table II) and in 47 and 7.9 per cent of the males, respectively.

FIG. 3.—Section of an adrenal gland of a mouse showing extensive accumulation of amyloid (Stage 3). A large part of the cortex has been replaced. × 90.

The extent of the brown degeneration was greatest in the adrenals of 2 of the cancer-resistant strains, N and NH, (Fig. 5). The adrenals of the A, C12I, and L strains (one high cancer, one medium cancer, and one cancer-resistant strain) showed the most extensive amyloid degeneration (Fig. 6).

The influence of the strain on the development of adrenal changes.—The adrenals of some mice of all the 11 strains showed brown degeneration. The adrenals of only 2 of 15 of the A strain of mice contained slight (Stage 1) accumulations of large brown cell masses. All of the adrenals of the female mice of the N, C57, and NH strains showed brown degeneration. In the females the incidence of brown degeneration in mice of the different strains ranged from 20 to 100 per cent (Fig. 5 and Table II). Likewise the different strains showed variations of 0 to 100 per cent in the incidence of amyloid degeneration (Fig. 6, Table II). The strains which in general exhibited the higher percentages of brown degeneration of the adrenals showed the lower incidences of amyloid (Figs. 5 and 6).

Influence of sex on the changes of the adrenal.—

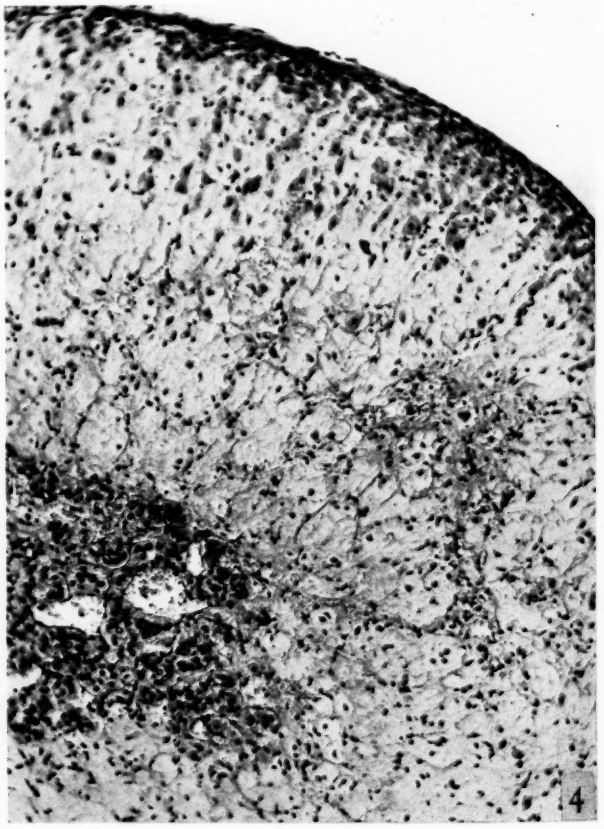


FIG. 4.—Section of an adrenal gland of a mouse. The cortex is largely replaced by an accumulation of amyloid (Stage 4). × 90.

The adrenals of 17 males from 4 of the susceptible strains and of 14 males of tumor-resistant strains were examined. Brown degeneration occurred in 29.4 per cent and 50 per cent of these animals, respectively. Five of the 6 males of the C3H strain constituted the 29.4 per cent in the tumor-susceptible group. Brown degeneration was not detected in the 11 males from the other 3 tumor-susceptible strains. The incidence of brown degeneration in the males of the tumor-susceptible strains was less than one-half of that of the females.

Amyloid degeneration occurred in approximately

equal percentages of males and females in both the cancer-susceptible and cancer-resistant groups.

Degenerative changes in the adrenal with advancing age.—The number of mice in the younger age groups was too small to determine the earliest age of appearance of degenerative changes. The adrenals of over 50 per cent of the female mice of the age groups 200 days or over showed brown degeneration. Amyloid degeneration occurred in the adrenals of female mice of all age groups. There was little evidence to indicate that the incidence of brown degeneration increased with age in the individual strains. Amyloid degeneration tended to occur in the older mice of the C57 and C3H strains but in the other strains there was no obvious age relationship. The extent of brown and amyloid degeneration tended to be greatest in mice of the older age groups in most strains. The adrenals of mice of the N strain of all ages showed marked brown degeneration (Table II).

Two mice lived over 700 days. One was from the N strain and showed brown degeneration (Stage 3) and the other from the L strain with no brown degeneration. Eight mice from the N, NH, and C57 strains lived from 600 to 700 days and all showed brown degeneration (Fig. 7). Other animals from these 3 strains which were killed at less than 400 days possessed adrenals with equally extensive lesions.

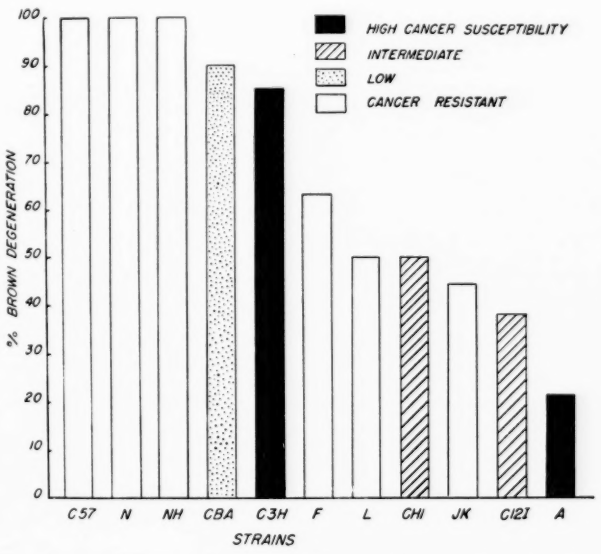


FIG. 5.—The incidence of brown degeneration in the adrenal glands and of mammary tumors in female mice of 11 strains.

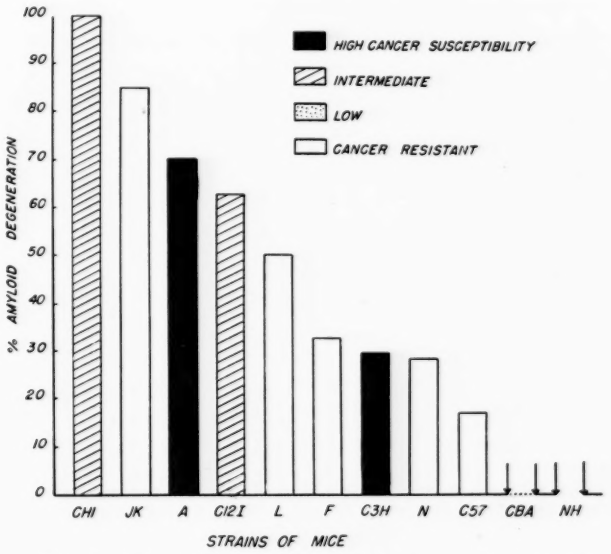


FIG. 6.—The incidence of amyloid degeneration in the adrenal glands and of mammary tumors in female mice of 11 strains.

DISCUSSION

The frequent occurrence of brown degeneration in females of the various strains studied in this investigation agrees with the observations on mice of other strains (2, 5, 8, 11). The number of animals in the different strains which showed this degenerative process was extremely variable, however. If the incidence in females alone is considered, it ranged from 20 to 100 per cent in the various strains. Brown degeneration occurred in the glands of over half the females from 8 strains.

No etiological relationship of brown degeneration to susceptibility to mammary carcinoma was apparent since this type of degeneration was not found exclusively in the glands of cancer-susceptible strains, was present in many cancer-susceptible mice which did not actually develop cancer, and the adrenal glands of a considerable number of mice which developed mammary cancer did not show brown degeneration. Moreover, brown degeneration was never far advanced or medullary in distribution in any of the females developing cancer.

The highest incidences of brown degeneration, 100 per cent, occurred in mice of 3 tumor-resistant strains, while the incidence was lower in mice of the tumor-susceptible strains. Kreyberg and Ecker (11) likewise noted that the susceptibility or resistance to mammary cancer in mice of their strains was not related to the incidence of brown degeneration of the adrenal glands.

The extent of the gland areas involved with brown degeneration was never great in animals of tumor-susceptible strains. In fact, this process had never advanced beyond the second stage in the adrenals of any of the animals which actually developed mammary tumors. Brown degeneration never extensively involved the medulla of the adrenals from animals of the various strains whether tumor-susceptible or not. In the majority of instances it was limited to the peri-

medullary zone, only its more advanced stages spreading outward to involve extensive portions of the cortex. Because medullary involvement with brown degeneration was lacking in the adrenals of all the mice from this series which had developed breast cancer, it is not evident how the interpretation of Cramer (5) could apply in these mice. The adrenal medulla, according to him, acts antagonistically against estrogenic substances thereby inhibiting their power of stimulating cancerous growth in the breast. This antagonistic effect would be eliminated if the adrenal medulla were rendered functionless by some such process as brown degeneration.

The early work (1-3) on brown degeneration offers another explanation. Brown degeneration was first reported as an incidental finding secondary to hypertrophy of the pituitary gland in estrogen-treated animals. These animals developed a state of hypopituitarism following hyperplasia of the hypophysial chromophobic cells. Degenerative changes were found not only in the adrenal glands of these animals but also in other organs, especially the gonads. This gonadal degeneration was probably due to the decreased amounts of, or total absence of, the hypophysial

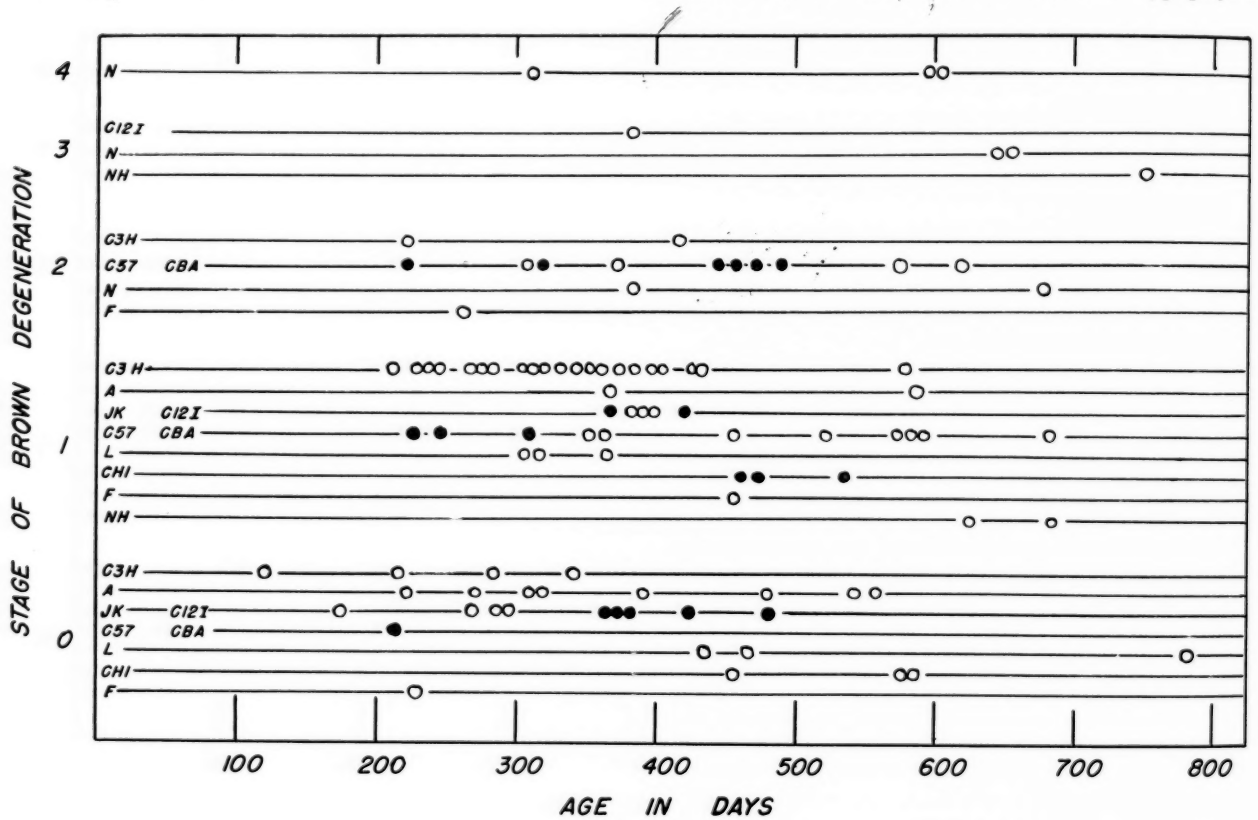


FIG. 7.—The extent of brown degeneration and the age distribution of its appearance in 99 female mice of 11 strains. (When 2 strains are grouped on the same line the solid dots designate the first strain listed on that line.)

An adequate explanation for the occurrence of brown degeneration must still be sought. Several possibilities have been suggested. Chronic disease or inanition may be a possible cause for the development of brown degeneration. It is possible that these conditions may accentuate this change but that it is the actual cause of the high incidence of brown degeneration among the well-fed, healthy animals of this series is doubtful.

The suggestion that brown degeneration may represent collections of phagocytic giant cells of the reticuloendothelial system which have appeared in the adrenal gland in response to the toxic action of estrogens, is not convincing (13). The cells which first show this brown pigment resemble very closely cortical parenchymal cells and not cells of the reticuloendothelium.

gonadotropic hormones. Gonadal degeneration of a similar type occurs in mice which have been hypophysectomized. Therefore, in estrogen-treated mice the adrenocorticotropic hormone, also secreted by the pituitary gland, may not be elaborated in effective amounts in the hyperplastic but hypo-functioning pituitary glands of the estrogen-treated animals. This belief is supported by the fact that extensive degeneration was observed in the adrenals of some hypophysectomized mice (12).

A final possibility, supported by the observations in this investigation, is that brown degeneration is a characteristic of certain inbred strains of mice. This is in entire agreement with the opinion of Kreyberg

and Ecker (11). This variation was definitely not related to the occurrence of mammary cancer and, therefore, the genetic factors responsible for the latter are presumably entirely separate and independent of the factor causing brown degeneration.

Environmental factors such as the general health of the animal, its age and sex, as well as deliberate experimentation (estrogenic treatment, hypophysectomy, starvation, etc.) may affect profoundly the development and appearance of this process which is already a part of the animal's hereditary make-up. For example, the injection of estrogenic substances caused a much earlier appearance and a more extensive brown degeneration in strains which normally exhibited a milder degree of this process much later in life (6, 13).

The lack of any definite relationship between brown degeneration to either mammary carcinomas or the tendency to develop such tumors was likewise evident with amyloid degeneration. Amyloid change was found in the glands of animals which had developed tumors, but it also occurred in mice without tumors. In addition, mammary carcinomas frequently appeared in animals whose adrenal glands showed no signs of amyloid change. The frequency and extent of this process showed no consistent relationship to the tumor-susceptibility in the various strains. Finally, extensive amyloid deposits were not found in the adrenal of a single female of the entire series which had developed a mammary carcinoma. No basis was evident for associating this particular adrenal degenerative change with the causation of mammary cancer.

An experimentally established explanation for the occurrence of this amyloid change is not available at present. The suggestion that amyloid change, like brown degeneration, is the result of inanition or of chronic disease may be plausible since it has been observed in similar conditions in man, but opposed to this suggestion is the fact that most of the animals of this series were in good general health, although many were quite aged.

Summary and Conclusions

1. Adrenal glands from 130 untreated mice, 31 males and 99 females, were examined. These animals were from 11 inbred strains of which 5 showed variable susceptibilities to the spontaneous development of mammary carcinoma. In the remaining 6 strains, mammary carcinomas never or rarely developed.

2. The structure of the adrenal glands and their variations from the usual histological appearance were observed and recorded. The occurrence of brown degeneration of Cramer (6) as well as a deposition of amyloid in the adrenal cortical region was studied.

3. Brown degeneration was found in 70.7 per cent of all the females and amyloid deposits in 39.3 per cent. The former varied in frequency in the females of the 11 different strains from 20 to 100 per cent; the latter from 0 to 100 per cent. There was no consistent relation between the frequency of either of these two processes and the potentiality of any strain to develop mammary carcinoma. The extent of degeneration was different in the various strains, but no animals which had developed mammary carcinomas showed more than a moderate stage of degeneration in their adrenals. On the other hand, the glands of many tumor-bearing animals showed no adrenal degeneration.

4. Amyloid degeneration was more extensive in the older animals.

5. The degenerative processes occurred less frequently, and were less extensive in males than in females.

6. The two degenerative processes could not be considered as either immediate or remote causes for the development of mammary carcinoma. Likewise, a common etiological factor responsible for the development of brown degeneration and mammary carcinoma was not evident.

References

1. Burrows, H. Pituitary Hyperplasia in a Male Mouse After the Administration of Estrin. Am. J. Cancer, **28**:741-745. 1936.
2. Burrows, H. Changes Induced by Estrogens in the Adrenals of Male Mice. J. Path. & Bact., **43**:121-126. 1936.
3. Cramer, W., and E. S. Horning. Experimental Production by Oestrin of Pituitary Tumors With Hypopituitarism and of Mammary Cancer. Lancet, **1**:247-249. 1936.
4. Cramer, W. The Effect of Oestrin on the Pituitary Gland. Lancet, **1**:1056-1057. 1936.
5. Cramer, W. The Hormonal Aetiology of Breast Cancer. Am. J. Cancer, **38**: 463-472. 1940.
6. Cramer, W., and E. S. Horning. On the Association Between Brown Degeneration of the Adrenals and the Incidence of Mammary Cancer in Inbred Strains of Mice. Am. J. Cancer, **37**:343-354. 1939.
7. Cramer, W., and E. S. Horning. Hormonal Relationships Between the Ovary and the Adrenal Gland. Lancet, **1**:192-197. 1939.
8. Dobrovolskaia-Zavadskaia, N., and P. Zephiroff. La surrénale dans les lignées de souris à potentialité cancérigène différente. Compt. rend. Soc. de biol., **128**:97-99. 1938.
9. Hett, J. Beobachtung an hungernden Tieren und nach Injektionen von Trypanblau. Ztschr. f. mikr.-anat. Forsch., **7**:403-420. 1926.
10. Kolmer, W. Zur vergleichenden Histologie, Zytologie und Entwicklsgeschichte der Saugernebenniere. Arch. f. mikr. Anat., **91**:1-79. 1918.
11. Kreyberg, L., and R. Ecker. Brown Degeneration of the Adrenal Gland and Breast Cancer in Mice. Vid-Akàd. Avh. I. M.-N. Kl., **3**:1. 1939.
12. Leblond, C. P., and W. O. Nelson. Etude histologique des organes de la souris sans hypophyse. Bull. d'histol. appliq. à la physiol., **14**:181-204. 1937.
13. Lacassagne, A., and A. Raynaud. Apropos de l'action de l'oestrine sur la surrénale: Les symplasme phagocytaires chez la souris. Compt. rend. Soc. de biol., **124**:1183-1186. 1937.
14. Whitehead, R. The Significance of Natural Variations in the Structure and Cortical Lipoid of the Mouse Suprarenal. Brit. J. Exper. Path., **12**:305-311. 1921.

The Influence of Estrogens on the Incidence of Tumors in Foster Nursed Mice*

John J. Bittner, Ph.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

(Received for publication March 21, 1941)

Results of experiments on laboratory animals have demonstrated that estrogenic hormones and the genetic constitution of the host are important contributive factors in the development of carcinoma of the breast. Since the work of Lacassagne (16) showing that this type of tumor may result following the injection of estrogens, important advances have been made. This subject has been reviewed by Gardner (13) and Loeb (18). The following is a summary of the results as given by Gardner:

> "The observations on mice with known incidences of tumor appearance indicate that intrinsic factors other than estrogens play a significant role in mammary carcinogenesis. In some strains of mice, however, the influence of these intrinsic factors may be largely dominated by the effects of estrogens administered for long periods. In other strains, characterized by very low incidence of spontaneous carcinogenesis, few mammary tumors have been obtained by administration of estrogens."

That there may be another influence functioning in the induction of tumors in estrogen-treated mice in addition to the hereditary and estrogenic factors, was indicated in the work of Twombly (21). In this experiment males of the C57 black stock which had been nursed by females of the cancer-susceptible R III strain received pellets of estrone. Mammary carcinoma resulted in 9 mice or 33 per cent of the total. The incidence of induced tumors of the breast has been less than 1 per cent in unfostered males of this strain as shown by Suntzeff, Burns, Moskop, and Loeb (20) and Haagensen and Gardner as reported by Twombly (21).

At the Third International Cancer Congress in 1939, in the first report on the influence of nursing, Twombly stated that males of the R III strain nursed by mothers of the C57 black strain showed a lower incidence of mammary cancer following treatment with estrogens than did the unfostered males. The final results have not been reported.

Experimental

The purpose of this experiment was to determine if the incidence of carcinoma of the breast resulting from the administration of crystalline estrogens, in pellet form, would approach the incidence of spontaneous tumors observed in breeding females of inbred stocks and hybrid generations. If similar ratios could be obtained breeding might be omitted with a saving of both time and space.

The animals injected were representatives of the inbred A, C3H (Z), and C57 black (B) strains. Breeding females of the B stock have a low incidence (17) and the others (1, 3, 4, 8-10) a high incidence of carcinoma of the mammary glands. Mice of the C3H stock fostered by females of the B strain are represented as "Z by B♀." The Ax mice were animals of the A stock descended from females fostered by mothers of the CBA (X) strain. Few of the breeding females had tumors (5, 8, 9). A female of the eighth generation of the Ax line gave rise to the AxT subline. This female developed a mammary tumor and her descendants have had a high incidence of mammary cancer (9). The other mice were hybrids produced by reciprocal matings between mice of the A and C57 black or B strains. The first letter used to represent the generation refers to the maternal strain. All the hybrids were nursed by or descended from females which had nursed mothers of the cancerous A or the noncancerous B strains.

Pellets of estrone [1] (average weight 2 mgm.) or estradiol benzoate [2] (average weight 1.5-2.0 mgm.) were implanted subcutaneously when the mice were from 4 to 6 weeks of age. The pellets were prepared in a press.

The results are tabulated in Table I by age periods following the time of injection. Mice dying of hyperestrinism before the appearance of the earliest tumor are not included. The experiment was terminated 227 days following the appearance of the last tumor. The observations on the mice of the second hybrid and backcross generations to the B stock are grouped according to the influence in the milk of the females on which they nursed.

The number of animals observed was 315 but the number of classes does not justify a detailed examina-

* This investigation was aided by grants from the National Cancer Institute.

[1] Parke, Davis & Co.

[2] The Schering Corporation.

tion of the results. With the exception of the males of the C3H strain, every other group of inbred mice nursed by females of the strains having a high incidence of spontaneous mammary tumors included some mice which gave rise to induced tumors. None of the estrogen-treated mice nursed by females of stocks with a low incidence of spontaneous tumors developed tumors regardless of their genetic constitution.

Few tumors appeared after 9 months following treatment. In some groups, as the males of the ABF1 generation receiving estradiol benzoate, the animals which died before this age showed a high incidence while the mice which survived longer than 9 months were free from tumors.

The results for mice of the inbred strains are summarized in Table II. Theoretically these animals are either susceptible or nonsusceptible for the inherited constitution for spontaneous tumors of the mammary glands.

TABLE I: RESULTS OBSERVED FOLLOWING THE IMPLANTATION OF PELLETS OF ESTRONE AND ESTRADIOL BENZOATE IN MICE

			Pellets of estrone					Pellets of estradiol benzoate					Estrone		Estradiol benzoate	
			Number cancerous or noncancerous by months *											Cancer per		Cancer per
Stock	Nursed by †	Sex	3-5	6-8	9-11	12-14	15-16	3-5	6-8	9-11	12-14	15-16	No.	cent	No.	cent
A	HC♀	♀	2/0	1/1	..	..	..	1/0	3/3	0/1	0/1	..	4	75	9	44
	HC♀	♂	1/1	1/0	0/1	..	..	1/0	0/1	..	0/1	..	4	50	3	33
Ax	LC♀	♀	..	0/9	0/3	..	0/1	0/1	0/8	0/3	0/7	..	13	0	19	0
	LC♀	♂	..	0/1	0/1	..	0/1	..	0/4	0/5	0/3	..	4	0	12	0
AxT	HC♀	♀	3/0	1/0	..	..	..	..	..	..	..	..	4	100	..	..
	HC♀	♂	..	1/0	0/1	..	..	..	..	..	..	..	2	50	..	..
Z (C3H)	HC♀	♀	..	1/0	2/2	..	..	..	..	..	..	..	5	60	..	..
	HC♀	♂	0/3	0/2	..	..	..	..	..	..	..	..	5	0	..	..
Z by B♀	LC♀	♀	0/5	0/2	0/1	..	..	..	..	..	..	..	8	0	..	..
	LC♀	♂	0/3	0/2	..	..	..	..	..	..	..	..	5	0	..	..
B (C57 black)	LC♀	♀	..	..	..	..	..	..	..	0/4	..	..	..	..	4	0
	LC♀	♂	..	..	..	..	..	..	0/1	0/1	0/5	..	..	..	7	0
ABF1 by A♀	HC♀	♀	..	..	..	..	..	0/2	..	0/1	0/1	..	..	..	4	0
	HC♀	♂	..	..	..	..	..	1/3	2/0	0/1	0/9	..	..	..	16	19
BAF1 by B♀	LC♀	♀	..	..	0/2	..	..	0/1	0/2	0/7	0/10	..	2	0	20	0
	LC♀	♂	..	0/3	0/4	0/3	0/2	0/1	0/5	0/6	0/4	..	12	0	16	0
F2 and BBC	HC♀	♀	1/6	2/14	0/4	0/3	0/2	0/2	1/4	..	0/2	0/1	32	9	10	10
	HC♀	♂	0/4	2/7	2/10	1/3	..	0/2	0/5	0/4	0/3	..	29	17	14	0
F2 and BBC	LC♀	♀	0/1	0/3	0/2	0/1	..	0/4	0/11	0/3	0/2	..	7	0	20	0
	LC♀	♂	0/2	..	0/2	0/3	0/5	0/2	0/5	0/4	0/8	..	12	0	19	0

* Numerators denote the number of mice developing breast cancer and denominators the number of mice dying without cancer during each age period.
† HC = high cancer. LC = low cancer.

TABLE II: SUMMARY OF OBSERVATIONS OF MICE OF INBRED STRAINS FOLLOWING THE ADMINISTRATION OF PELLETS OF ESTROGENS. (AX MICE ARE FOSTERED ANIMALS OF THE A STOCK, THE AXT LINE IS A SUBLINE OF THE AX GROUP HAVING A HIGH INCIDENCE OF MAMMARY TUMORS, AND THE Z BY B♀ ANIMALS ARE MICE OF THE C3H STRAIN NURSED BY FEMALES OF THE C57 BLACK OR B STOCK.)

		Estradiol benzoate				Estrone			
		Females		Males		Females		Males	
Stock	Incidence of spontaneous carcinoma	No.	Induced cancer incidence, per cent	No.	Induced cancer incidence, per cent	No.	Induced cancer incidence, per cent	No.	Induced cancer incidence, per cent
A	High	9	44	3	33	4	75	4	50
Ax	Low	19	0	12	0	13	0	4	0
AxT	High	..	..	..	..	4	100	2	50
Z (C3H)	High	..	..	..	..	5	60	5	0
Z by B♀	Low	..	..	..	..	8	0	5	0
B	Low	4	0	7	0	..	..	..	..

DISCUSSION

The results obtained in these experiments would not suggest the use of estrogenic hormones as a substitute for breeding in genetic studies on the incidence of mammary carcinoma. As a considerable proportion of the mice died of hyperestrinism, it is possible that the dosage was too great to be tolerated by some mice. On the other hand, many animals recovered from the

effects of the treatment and survived several months longer than did the oldest cancerous mouse.

The results may be of interest because mammary tumors were not induced in any animal which had been nursed by females of strains which had a low incidence of spontaneous tumors. Carcinoma of the mammary gland resulted in some mice, males and females, of the groups nursed by potentially cancerous females, confirming the work of others (11, 14, 15, 19, 20).

The injection of fostered mice of a low cancer strain by Twombly (21) raised the question if an inherited susceptibility for spontaneous mammary tumors was needed for the induction of tumors by estrogenic hormones. One per cent of the breeding females of the strain which he used have breast tumors (17) and when fostered by mothers of strains having a high incidence, the proportion is approximately 10 per cent (1, 6, 8).

These inconclusive results would tend to indicate that an active influence obtained in the milk by nursing females of cancerous strains may be of greater importance in the production of induced tumors by the administration of estrogenic hormones than is an inherited susceptibility for spontaneous mammary tumors. An excessive amount of hormone may be able to overcome the lack of the intrinsic factor when operating with an active milk influence. Few tumors are induced, however, when the influence in the milk is inactive although the host may be genetically susceptible for the development of spontaneous tumors.

As it has been noticed that the active influence in the milk may arise *de novo* or by changes in the inactive influence (9), it is possible that some mice of a strain or sublines of the same strain which have a low incidence of spontaneous mammary carcinoma may have an active milk influence. From Twombly's results, tumors would be expected in such animals.

To emphasize this point it should be mentioned that Gardner (12) induced mammary carcinoma in mice of both sexes of the CBA stock but estrogen-tested mice of the A stock nursed by females of the CBA (called X) strain have been resistant to mammary tumors.

By foster nursing it should be possible to determine if the influence in the milk is active or inactive in mice of any strain or subline.

Summary

The administration of estrogenic hormones may result in the induction of mammary tumors in mice which have: (a) an inherited susceptibility for spontaneous tumors and an active milk influence; (b) an active milk influence without an inherited susceptibility for the development of spontaneous tumors.

When the influence in the milk has been proved to be inactive few estrogenic tumors have been reported.

REFERENCES

1. Andervont, H. B., and W. J. McEleney. The Influence of Foster Nursing upon the Incidence of Spontaneous Breast Cancer in Strain C3H Mice. Pub. Health Rep., **54**:1597-1603. 1939.
2. Andervont, H. B. The Influence of Foster Nursing upon the Incidence of Spontaneous Mammary Cancer in Resistant and Susceptible Mice. J. Nat. Cancer Inst., **1**:147-153. 1940.
3. Bittner, J. J. The Breeding Behavior and Tumor Incidence of a Black Agouti Stock of Mice. Am. J. Cancer, **25**:614-620. 1935.
4. Bittner, J. J. Breast Cancer in Breeding and Virgin "A" and "B" Stock Female Mice and their Hybrids. Pub. Health Rep., **54**:1113-1118. 1939.
5. Bittner, J. J. Breast Cancer and the Pedigree Relationship of Fostered A Stock Mice. Pub. Health Rep., **54**:1642-1651. 1939.
6. Bittner, J. J. The Influence of Transplanted Normal Tissue on Breast Cancer Ratios in Mice. Pub. Health Rep., **54**:1827-1831. 1939.
7. Bittner, J. J. Further Studies on Active Milk Influence in Breast Cancer Production in Mice. Proc. Soc. Exper. Biol. & Med., **45**:805-810. 1940.
8. Bittner, J. J. Breast Cancer in Mice as Influenced by Nursing. J. Nat. Cancer Inst., **1**:155-168. 1940.
9. Bittner, J. J. Changes in the Incidence of Mammary Carcinoma in Mice of the A Stock. Cancer Research, **1**:113-114. 1941.
10. Bittner, J. J. The Variability of Incidence of Mammary Cancer in Mice of an Inbred Strain. Cancer Research, **1**:115-120. 1941.
11. Burns, E. L., and J. R. Schenken. Quantitative Studies on Relationship Between Estrogen and Mammary Gland Carcinoma in Strain C3H Mice. Proc. Soc. Exper. Biol. & Med., **43**:608-610. 1940.
12. Gardner, W. U. Influence of Estrogenic Hormones on Abnormal Growth. Science, **85**:Suppl. 67-75. 1937.
13. Gardner, W. U. Estrogens in Carcinogenesis. Arch. Path., **27**:138-170. 1939.
14. Gardner, W. U., G. M. Smith, E. Allen, and L. C. Strong. Cancer of the Mammary Gland Induced in Male Mice Receiving Estrogenic Hormone. Arch. Path., **21**:265-272. 1936.
15. Gardner, W. U., G. M. Smith, L. C. Strong, and E. Allen. Experimental Production of Malignant Growths in Mice by Estrogenic Chemicals. J. A. M. A., **107**:656-657. 1936.
16. Lacassagne, A. Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine. Compt. rend. Acad. d. sc., **195**:630-632. 1932.
17. Little, C. C., W. S. Murray, and A. M. Cloudman. The Genetics of Non-epithelial Tumor Formation in Mice. Am. J. Cancer, **36**:431-450. 1939.
18. Loeb, L. The significance of hormones in the origin of cancer. J. Nat. Cancer Inst., **1**:169-195. 1940.
19. Shimkin, M. B., and H. G. Grady. Carcinogenic Potency of Stilbestrol and Estrone in Strain C3H Mice. J. Nat. Cancer Inst., **1**:119-128. 1940.
20. Suntzeff, V., E. L. Burns, M. Moskop, and L. Loeb. The Effects of Injections of Estrin on the Incidence of Mammary Cancer in Various Strains of Mice. Am. J. Cancer, **27**:229-245. 1936.
21. Twombly, G. H. Breast Cancer Produced in Male Mice of the C57 (Black) Strain of Little. Proc. Soc. Exper. Biol. & Med., **44**:617-618. 1940.

Studies on Prostatic Cancer

I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate*

Charles Huggins, M.D., and Clarence V. Hodges, M.D.

(From the Department of Surgery, the University of Chicago, Chicago, Illinois)

(Received for publication March 22, 1941)

Carcinoma of the prostate gland is peculiarly favorable for endocrine investigation since frequent serial observations of the activity of phosphatases in serum were found to provide objective indices of activity of the neoplasm when the enzymes were increased in amount above normal. In the present paper data are given for the values of serum phosphatases in carcinoma of the prostate and in normal men. We shall demonstrate that the acid phosphatase of serum is reduced in metastatic carcinoma of the prostate by decreasing the activity of androgens through castration or estrogenic injections and that this enzyme is increased by injecting androgens. We have been unable to find previous observations indicating any relationship of hormones to carcinoma of the prostate gland.

An enzyme capable of hydrolyzing phosphoric esters was discovered by Grosser and Husler (4) in intestinal mucosa and kidney. Robison (16) found that this enzyme was particularly high in activity in growing bone and cartilage and that its activity was greatest at pH 9 to 9.5. This "alkaline phosphatase" was found by Kay (9) to be increased in the serum in certain bone diseases including metastasis of neoplasms to bone and later work has shown that among these conditions is carcinoma of the prostate.

Davies (3) and Bamann and Riedel (1) discovered that there occurs in the spleen and kidney of swine and cattle, in addition to the alkaline phosphatase, a phosphatase with an activity maximum at pH 4.8. An enzyme believed to be identical with this "acid phosphatase" was found by Kutscher and Wolbergs (11) to be present in very large amount in the human prostate gland. This finding of great activity of acid phosphatase in the prostate gland was confirmed and extended to include prostatic cancer by Gutman, Sproul, and Gutman (7). The serum of certain patients with disseminated prostatic carcinoma was found by Gutman and Gutman (6) and Barringer and Woodard (2) to exhibit increased acid phosphatase activity. Robinson, Gutman, and Gutman (15) summarized the acid phosphatase activity levels of 44 patients with carcinoma of the prostate. They concluded that a marked rise in acid phosphatase in serum is associated with the appearance or spread of roentgenologically demonstrable skeletal metastases and implies dissemination of the primary tumor and thus is of unfavorable prognostic significance.

* This investigation was aided by a grant from the Committee on Research in Problems of Sex, the National Research Council.

Methods and Materials

The phosphatase activity of serum was determined by the method of King and Armstrong (10) using 0.005 M disodium monophenylphosphate as substrate. The buffers used were 0.05 M barbital-sodium at pH 9.3, and 0.1 M Sörensen's citrate-HCl or Walpole's 0.2 N sodium acetate-acetic acid buffers at pH 5. All serums were tested in duplicate and were added directly to buffer-substrate solutions without dilution; they were incubated at 37.5° C. for 30 minutes. Precautions were observed that all solutions were at this temperature before testing. Blanks were run by adding the protein precipitant to the buffer-substrate solution before adding serum. Colorimetric procedures were carried out with the Evelyn photoelectric colorimeter using a 6600 Å filter. The results are expressed in King and Armstrong units, a unit being defined as that degree of phosphatase activity which at pH 9.3 (or pH 5.0, respectively) and 37.5° C. will liberate 1 mgm. of phenol from the specified buffer-substrate solution in one-half hour.

Phosphatase determinations at pH 5 and 9.3 were made on the serum of 40 normal men, of 21 men with benign prostatic hypertrophy, and of 47 men with carcinoma of the prostate. The diagnosis of carcinoma of the prostate gland was derived from one or more of the following procedures: rectal palpation, cystoscopic examination, transurethral resection with microscopic examination, or roentgenologic evidence of skeletal metastases. Necropsy was obtained in 2 cases. All patients had x-ray studies of the bony pelvis.

Eight patients who had carcinoma of the prostate with skeletal metastases and with moderate or great elevation of acid phosphatase of serum values above 20 units in 100 cc. were selected for intensive study in the hospital. Each patient also had elevation of alkaline phosphatase in the serum. Both of these enzymes were determined on the serum 3 times weekly for many weeks. Bilateral castration was carried out in all. Five patients were injected with stilbestrol, 1 mgm.

daily, or estradiol benzoate, 1.66 to 3.32 mgm. daily, before castration. Three patients were injected with testosterone propionate,[1] 25 mgm. daily, in sesame oil.

The phosphatases of cerebrospinal fluid in 3 cases and of spermatocele fluid in 1 case were determined concurrently with those of the serum on 1 occasion. *In this paper, units of enzyme refer to 100 cc. of the specified body fluid.*

In 4 normal adult male dogs, serial observations of the phosphatases of the serum were made during 1 week before and 4 weeks after bilateral castration. Castration was done under ether anesthesia.

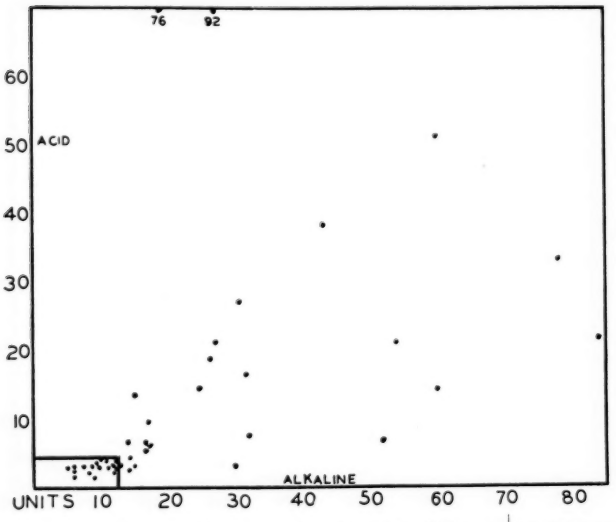


FIG. 1.—Scatter diagram of acid and alkaline phosphatase values in serum in carcinoma of the prostate. Ordinates, acid phosphatase; abscissae, alkaline phosphatase.

RESULTS

Values in carcinoma of the prostate and in controls. The values with standard deviation in the control groups were: in the serum of 40 normal men, acid phosphatase 3.25 ± 1.37 units, alkaline phosphatase 8.15 ± 1.74 units. The upper limit for acid phosphatase is slightly higher than that of Gutman and Gutman (6) who used citrate buffer, whereas most of our determinations were made with acetate buffering. The nature of the buffer is not the cause of the difference obtained, however, since determinations made in parallel using citrate and acetate buffer yielded identical results.

The values in 47 cases of carcinoma of the prostate with and without demonstrable metastases are shown in Fig. 1. Of these 47 patients, 23 had acid phosphatase below 4.5 units and alkaline phosphatase below 12.5 units; of the remaining 24 patients all had elevation of alkaline phosphatase above 12.5 units, and 21 had acid phosphatase values above 5 units.

Correlation between roentgenologic evidence of skeletal metastases and serum phosphatases.—Positive x-ray evidence of metastasis of prostatic carcinoma to the bony pelvis was obtained in 25 cases. Both acid and alkaline phosphatase activities of the serum were increased above normal in 19 cases. Alkaline phosphatase alone was increased in 2 cases while acid phosphatase was within normal limits, and neither enzyme was increased in 4 cases. There was no evidence of metastasis on x-ray examination of the pelvis of 20 patients; in 16 men both acid and alkaline phosphatase were normal; in 3 men alkaline phosphatase was elevated but in none of this group was the acid phosphatase increased in amount. No deductions could be made from enzyme study of the serum with respect to the osteoplastic or osteolytic nature of the metastatic lesion.

Effect of castration. Acid phosphatase.—In each of 8 patients with prostatic cancer this activity rapidly decreased following bilateral orchiectomy.

Following operation, in the case of P. R., there was a fall from 26 to 5 units in 7 days (Fig. 2); in the case of J. R. a decrease from 35 to 3 units in 9 days (Fig. 3); in the case of O. A. there was a fall from 25 to 5 units in 12 days; in the case of C. B. a decrease from 39 to 9 units in 12 days.

Following castration the decrease usually persisted in a range slightly above or below the upper limits of the normal values; it was maintained at this value with slight fluctuation during the period of observation which in the longest case has been 180 days.

The values observed in the case of P. R. varied from 2.5 to 7.5 units during 150 days; in the case of J. R. between 3 and 6 units in 104 days; in the case of C. B. between 7 and 9 units in 125 days; and in M. M. the values fluctuated between 9 and 15 units per 100 cc. in 180 days.

In one patient, S. R., acid and alkaline phosphatase levels of serum were 90 and 30 units respectively 163 days after orchiectomy. No other enzyme studies were made on this man, who was the only patient with high enzyme levels after orchiectomy.

In one patient, J. F., with benign prostatic hypertrophy who was subjected to castration, as previously reported by Huggins and Stevens (8), acid and alkaline serum phosphatases were 3 and 9.25 units, respectively, 2 years after orchiectomy.

In 4 dogs, the values of the serum phosphatases were within the range found in normal men; bilateral orchiectomy resulted in no essential changes in their levels.

Alkaline phosphatase.—A rise was always observed in the value of this enzyme following castration in this series of patients. This rise was then succeeded by a decrease to or towards the normal value.

In the case of J. R., the value at the time of castration was 19 units. The maximum value, 41 units, was attained 39 days following orchiectomy, and at 104 days the value was 20 units. In the case of P. R. the precastration value was 22 units, the maximum value found was 27 units 10 days after castration, and

[1] The authors are indebted to Dr. Erwin Schwenk and the Schering Corporation for generous donations of estradiol benzoate and testosterone propionate and to Dr. J. A. Morrell of E. R. Squibb & Sons for the stilbestrol used.

this value decreased to 9 units at 150 days. In the case of C. B. the precastration value was 82 units, the maximum rise to 94 units was seen 12 days following orchiectomy, and the last value obtained was 35 units 125 days following testis removal.

Effect of estrogen injection.—Estrogen was given during periods of 8 to 23 days; all patients developed small palpable areas of subareolar breast tissue. The results were similar to those observed following castration; namely, a sharp decrease of acid phosphatase accompanied by an increase of alkaline phosphatase in the serum.

The patient P. M. was injected with 7 mgm. stilbestrol and 46.5 mgm. estradiol benzoate in 27 days. The acid phosphatase of his serum decreased from 50 to 19.5 units while the alkaline phosphatase rose from 55 to 95.5 units. J. C. received 8 mgm. stilbestrol in 8 days. His acid phosphatase fell from 32 to 4.5 units while the alkaline phosphatase rose from 27.5 to 64.5 units. O. A. received 30 mgm. stilbestrol in 23 days (Fig. 4). Acid phosphatase of his serum decreased from 48 to 4.5 units while alkaline phosphatase increased from 23 to 41.5 units.

Effect of androgen injection.—Testosterone propionate caused an increase of serum acid phosphatase above the pre-injection level in these patients. Following the cessation of injections, there was a decline to the preliminary level. In one patient this decrease was succeeded by a marked increase in acid phosphatase. There were no changes in alkaline phosphatase during the period of injection, but a rise occurred subsequent to treatment.

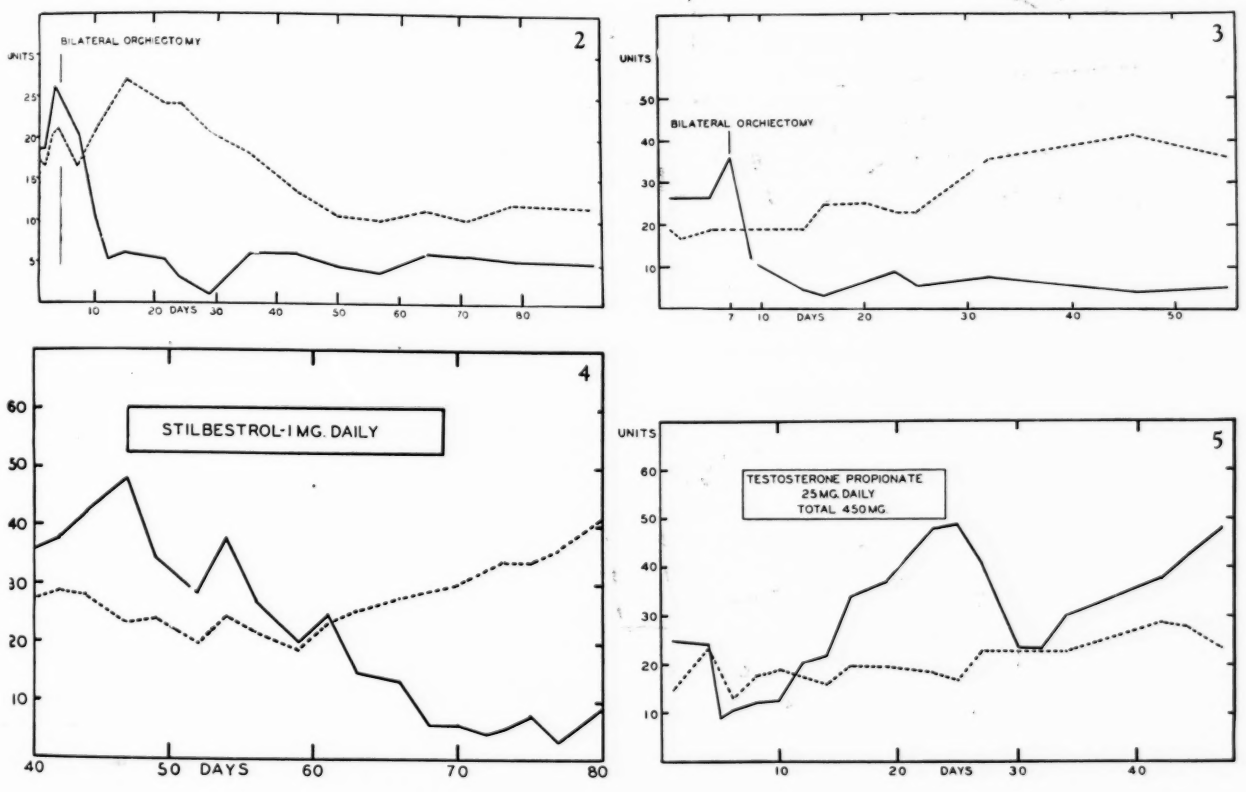


FIG. 2.—The effect of castration on serum phosphatases in metastatic carcinoma of the prostate in the case of P. R. Acid phosphatase ————; alkaline phosphatase – – – – –.

FIG. 3.—The effect of castration on serum phosphatase values in metastatic carcinoma of the prostate in the case of J. R. Acid phosphatase ————; alkaline phosphatase – – – – –.

FIG. 4.—The effect of estrogen injection, 1 mgm. stilbestrol for 23 days, on serum phosphatases in metastatic carcinoma of the prostate in the case of O. A. Acid phosphatase ————; alkaline phosphatase – – – – –. Ordinates, units per 100 cc. of serum; abscissae, time in days.

FIG. 5.—The effect of androgen injection, testosterone propionate, 25 mgm. daily for 18 days, on serum phosphatases in metastatic carcinoma of the prostate in the case of O. A. Acid phosphatase ————; alkaline phosphatase – – – – –.

In the case of C. B., previously castrated, injected with 25 mgm. testosterone propionate daily for 15 days, acid phosphatase rose from 9 to 27 units; cessation of androgen injection was followed by a decrease to 9 units 9 days following injection. In the case of O. A., injected with 25 mgm. testosterone propionate daily for 18 days, acid phosphatase rose from 11 units to a high value of 48.75 units reached the day following completion of androgen injection. This value decreased to 23.5 units 6 days following cessation of androgens, whereupon it rose to 46 units in the next 27 days (Fig. 5).

Distribution of phosphatases in body fluids in carcinoma of the prostate.—The phosphatases were found

to be low in cerebrospinal fluid and in spermatocele fluid although the values were concurrently high in blood serum.

In the case of P. M., acid and alkaline phosphatases of the spinal fluid were 0.75 and 0 units at a time when these enzymes in the serum were 51 and 64 units respectively. Similarly in O. A., acid and alkaline phosphatases of spinal fluid were 0 and 0 at a time when the findings in serum were 23 and 22 units respectively. In the case of J. C., acid and alkaline phosphatases of spinal fluid were 0 and 1.5 units when these levels in serum were 19.5 and 46 units respectively. In the case of C. R., acid and alkaline phosphatases of spermatocele fluid were 0 and 1.5 when the serum levels were 49 and 62 units, respectively.

Discussion

In disseminated carcinoma of the prostate the lability of acid phosphatase in serum as influenced by androgen supply or withdrawal indicates that this malignancy is influenced by the activity of male hormones in the host. With respect to serum enzymes, prostatic cancer is inhibited by decreasing androgenic hormonal function and activated by increasing the androgens. Moreover, our data, as yet unpublished, show that inhibition is not limited to the enzyme content of serum.

Certain bone diseases, as shown by Kay (9), are associated with increased alkaline phosphatase activity of serum and Roberts (14) discovered that certain liver diseases cause a similar increase. At the present time elevation of alkaline phosphatase of serum is regarded as indicating only bone or hepatic disease. There is no evidence to show that hepatic damage occurs with frequency in carcinoma of the prostate while extensive bone involvement by metastasis is often observed. It can be assumed with little risk that elevation of alkaline phosphatase of serum in carcinoma of the prostate is usually due to bone lesions, in the absence of gross evidence of hepatopathy.

Marked increases in acid phosphatase in serum have been observed only in prostatic carcinoma. Robinson, Gutman, and Gutman (15) found slight but definite elevations of this enzyme (the highest 8.7 units) in osteitis deformans and several other diseases where the bones were involved. The data in the present paper indicate that carcinoma of the prostate with skeletal metastasis is sometimes present with serum acid phosphatase values within the normal range. However, we found that when there was a marked rise of acid phosphatase (above 10 units) disseminated prostatic carcinoma was always present.

Gutman and Gutman (6) observed that there was no correlation between the height of serum acid and alkaline phosphatases in carcinoma of the prostate, and in general our data agree with their findings. This lack of correlation can now be explained. The elevated phosphatase levels in carcinoma of the prostate are due to the action of two different types of cells, prostatic epithelium and osteoblasts. The activity of prostatic epithelium (as deduced from the height of serum acid phosphatase) in disseminated cancer of the prostate depends at least in part on the degree of activity of androgens present in the body, whereas elevation of alkaline phosphatase is dependent on increased osteoblastic function as a result of invasion of the bones by tumor. It is thus apparent that increased acid and alkaline phosphatase activities in serum may be considered separately since they indicate activity of different types of cells. The function of those cells contributing to the greater part of the moiety whose maximum occurs at pH 5 is dependent on androgen levels, and can be increased or decreased by adjusting the male sex hormone level.

Having established a relationship of androgenic hormones to carcinoma of the prostate, the findings in the case of O. A. (Fig. 5) can be interpreted. In this man the serum acid phosphatase had been greatly increased by androgen injections and, following cessation of androgen, the enzyme decreased for 1 week when it was succeeded by an abrupt spontaneous secondary rise. The decrease following cessation of androgen injection may be assumed to be the result of the depression of gonadal activity caused by androgen injections as indicated by Moore and Price (12). The subsequent rise is explained by postulating that the cancer cells had been functionally expanded by androgen and therefore responded in an augmented way to hormones produced by the recovering testis.

Castration caused a marked decrease of serum acid phosphatase but in some cases the enzyme level did not reach normal while in others it lay in a range at the upper limit of normal values. Much proof has been adduced that androgen is produced in extragonadal loci especially in the suprarenal gland. The most ready explanation for variations in serum acid phosphatase levels following orchiectomy as well as the failure of some values to reach the normal range may be postulated by assuming varying amounts or activity of androgens produced in extragonadal sources in different individuals. It must be pointed out however that urinary assays of hormone excretion were not done in this study. From the evidence one cannot exclude the possibility that foci of metastatic carcinoma as well as the original site might affect the levels of phosphatase and so maintain this enzyme at an abnormally high range even though there were no androgens produced in extragonadal areas. Against this alternative explanation is the fact that atrophic prostatic tissue contains merely traces of acid phosphatase as Gutman and Gutman (5) found in the infantile human prostate gland.

None of the phosphatases has been isolated as yet

in a pure state. The findings of none or small amounts of these enzymes in cerebrospinal or spermatocele fluids show that the enzymes are molecules which do not cross certain membranes readily from the blood and are not inconsistent with the evidence of Kutscher and Pany (12) that phosphatase is a protein.

Summary and Conclusions

1. The content in serum of phosphatase with activity maximum at pH 5 in normal men and in men with benign prostatic hypertrophy was 3.25 ± 1.37 King and Armstrong units in 100 cc. Serum phosphatase with activity maximum at pH 9.3 in this control group was 7.9 ± 2.1 units in 100 cc.

2. In a group of 25 men with roentgenologic evidence of metastatic carcinoma to the bony pelvis, both acid and alkaline phosphatases were increased above normal in 19 cases, only alkaline phosphatase was increased in 2 cases, and both values were within normal limits in 4 cases. Metastatic carcinoma of the prostate may be present in bones when the phosphatases of serum are normal, but when acid phosphatase is present in activity greater than 10 units in 100 cc., disseminated prostatic cancer is present.

3. In prostatic cancer with marked elevation of acid phosphatase, castration or injection of large amounts of estrogen caused a sharp reduction of this enzyme to or towards the normal range. Alkaline phosphatase values rose following castration and then decreased, but more slowly than acid phosphatase. In certain cases, these values reached and were maintained in a normal range during the period of observation, 180 days, while in other patients the values were slightly above normal.

4. In 3 patients with prostatic cancer, androgen injection caused a sharp rise of serum acid phosphatase. In 1 case following cessation of androgen there was a decrease of the acid phosphatase followed by a secondary spontaneous rise.

5. Acid phosphatase does not readily cross membranes from the blood, since it was present only in traces in cerebrospinal and spermatocele fluids when concurrent serum level was high.

6. Prostatic cancer is influenced by androgenic activity in the body. At least with respect to serum phosphatases, disseminated carcinoma of the prostate is inhibited by eliminating androgens, through castration or neutralization of their activity by estrogen injection. Cancer of the prostate is activated by androgen injections.

References

1. Bamann, E., and E. Riedel. Ueber das Vorkommen zweier durch das pH Wirkungsoptimum unterscheidbaren Phosphoesterasen in tierischen Organen. Ztschr. f. physiol. Chem., **229**:125-150. 1934.
2. Barringer, B. S., and H. Q. Woodard. Prostatic Carcinoma with Extensive Intraprostatic Calcification. Tr. Am. A. Genito-Urin. Surgeons, **31**:363-369. 1938.
3. Davies, D. R. Phosphatase Activity of Spleen Extracts. Biochem. J., **28**:529-536. 1934.
4. Grosser, P., and J. Husler. Ueber das Vorkommen einer Glycerophosphatase in tierischen Organen. Biochem. Ztschr., **39**:1-5. 1912.
5. Gutman, A. B., and E. B. Gutman. "Acid" Phosphatase and Functional Activity of the Prostate (Man) and Preputial Glands (Rat). Proc. Soc. Exper. Biol. & Med., **39**:529-532. 1938.
6. Gutman, A. B., and E. B. Gutman. An "Acid" Phosphatase Occurring in the Serum of Patients with Metastasizing Carcinoma of the Prostate Gland. J. Clin. Investigation, **17**:473-478. 1938.
7. Gutman, E. B., E. E. Sproul, and A. B. Gutman. Significance of Increased Phosphatase Activity at the Cite of Osteoplastic Metastases Secondary to Carcinoma of the Prostate Gland. Am. J. Cancer, **28**:485-495. 1936.
8. Huggins, C., and R. E. Stevens. The Effect of Castration on Benign Hypertrophy of the Prostate in Man. J. Urol., **43**:705-714. 1940.
9. Kay, H. D. Plasma Phosphatase in Osteitis Deformans and in Other Diseases of Bone. Brit. J. Exper. Path., **10**: 253-256. 1929.
10. King, E. J., and A. R. Armstrong. Convenient Method for Determining Serum and Bile Phosphatase Activity. Canad. M. A. J., **31**:376-381. 1934.
11. Kutscher, W., and H. Wolbergs. Prostataphosphatase. Ztschr. f. physiol. Chem., **236**:237-240. 1935.
12. Kutscher, W., and J. Pany. Prostataphosphatase. Ztschr. f. physiol. Chem., **255**:169-189. 1938.
13. Moore, C. R., and D. Price. Some Effects of Synthetically Prepared Male Hormone (Androsterone) in the Rat. Endocrinology, **21**:313-329. 1937.
14. Roberts, W. M. Blood Phosphatase and van den Bergh Reaction in Differentiation of Several Types of Jaundice. Brit. M. J., **1**:734-738. 1933.
15. Robinson, J. N., E. B. Gutman, and A. B. Gutman. Clinical Significance of Increased Serum "Acid" Phosphatase in Patients with Bone Metastases Secondary to Prostatic Carcinoma. J. Urol., **42**:602-618. 1939.
16. Robison, R. The Possible Significance of Hexosephosphoric Esters in Ossification. Biochem. J., **17**:286-293. 1923.

Physiological Age as a Basis for the Comparison of Strains of Mice Subject to Spontaneous Mammary Carcinoma

William S. Murray, Sc.D., and Joseph G. Hoffman, Ph.D.

(From the New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y., Burton T. Simpson, M.D., Director.)

(Received for publication February 10, 1941)

Investigators who have attempted to explain the transmission of the disposition to neoplastic growth have been hindered by the inability to determine accurately the amount of cancer inherent in a stock. This inability is reflected in the variety of computations which have been used in the solution of this problem, in the conflicting interpretations of data which have been advanced, and in the general confusion surrounding the subject. Two factors contribute to this confusion: (a) the methods of computing the data may be erroneous within themselves, (b) the data as computed for more than one stock may not be mathematically comparable.

With the accumulation of observations upon a number of homozygous stocks, it has become increasingly clear that all of the strains thus far developed are subject to development of tumors in greater or lesser degree. It is also clear that the incidence of these tumors depends markedly upon the ages the mice attain. Korteweg (2) has pointed out that the disposition to have spontaneous tumors is measured not only by the fact that tumors appear but also by the age at which they occur. Murray (5) has presented preliminary calculations in which he compares the rates of cancer incidence when computed according to chronological age and physiological age.[1]

It is the purpose of this paper to compare the prevalent methods of computing cancer rates in mice, to propose a method of calculation in which corrected rates are treated as a function of physiological age, and to point out some of the advantages of this method.

Sources of Material

Data on 3 unrelated inbred strains of mice are used in these computations. They will be designated as (a) dilute brown, (b) Bittner albino, and (c) Marsh albino.

The dilute brown animals tabulated here are the breeding females of the Murray strain of the well-known Little Dba stock. They comprise the animals raised between 1925 and 1936 at the University of Michigan and the Roscoe B. Jackson Memorial Laboratory. A detailed report of the breeding behavior of this stock was published in 1934 (3).

The distribution designated as Bittner albinos was first published by Bittner and Murray (1) in a comparative study of 4 lines of mice with high tumor incidence. They comprise a summation of the mice listed in Tables III and V of that publication and should not be confused with tabulations of Bittner's present "A" stock.

The animals listed here as Marsh albinos are the breeding females of a strain developed in this laboratory by M. C. Marsh. The tabulation used here is a composite of animals raised by Marsh prior to his death in 1936 and by us since that time. A detailed description of the origin of this stock was published in 1938 (4).

Methods of Care

The methods of care used in maintaining these stocks were very similar. However, the types of food which they received varied. The dilute browns were fed a diet of hemp and canary seed, supplemented by whole raw milk and lettuce, while at Ann Arbor and, for a short time, at Bar Harbor. They were then changed to a ration of Purina Fox Chow and water.

The Bittner albino stock used here was about equally divided between mice which were fed an oatmeal diet and those which were fed Fox Chow.

The Marsh albinos were equally divided between animals which were fed a balanced ration prepared by Marsh and others which were fed Fox Chow.

Methods of Measuring Tumor Incidence

The most frequently used measure of the amount of cancer in a strain or experiment is probably the simple ratio or percentage. This is undoubtedly a

[1] The measure of age here designated as "physiological age" has been defined by Pearl (7a) as the "equivalent life span." Rahn (8, 9) has called it the "standardized time" in his studies on mortality.

carry over of the system used by geneticists in making distributions of the segregation of morphological characters. Since, however, cancer is essentially a disease of middle and old age, and since the segregates cannot be classified immediately, this measure is not entirely satisfactory for a problem of this type. This method is illustrated by the following summary taken from Tables IV and V.

These percentages may be corrected by considering only those animals which lived to the age at which cancer first appeared (Table II).

It is evident that this type of calculation is limited in the amount of information which it gives. It is unsatisfactory because it provides no information on: (a) the changing incidence of tumors as age advances and, (b) the relative opportunity of the various strains to have cancer. It may be misleading in that it gives the impression that 2 different strains of mice have the same degree of susceptibility to spontaneous cancer when they show the same percentages of tumors.

TABLE I: METHOD OF SIMPLE RATIOS

Stock	No. in experiment	No. with cancer	Percentage of cancer
Dilute brown	3,241	2,091	64.5
Bittner albino	730	526	72.0
Marsh albino	1,350	1,031	76.4

A further correction may be introduced by computing the percentages of cancer developing among animals which live to the beginning of each age group (Table III). It is obvious that mammary tumors are much more prevalent among the dilute browns during the older age periods and that the relationship of the incidence of cancer as expressed by the simple percentages of the first 2 tables is subject to question. Although the figures presented in this table demonstrate the variations in these stocks, it is difficult to determine from it which age shall be selected as being representative of these 2 strains.

METHOD OF CORRECTED RATES

In order to overcome the objections listed above, Murray and Little (9) resorted to a second method of computation; namely, the calculation of corrected rates (7b) with the aid of standard populations.[2]

[2] The formula employed in our calculations of corrected cancer rates is:

$$R = \frac{\Sigma L_s \times R_{cs}}{\Sigma L_s}$$

R is the corrected cancer rate.

L_s is the number of mice of age s in the standard population.

R_{cs} is the cancer rate per 10,000 population at age s in the strain of mice for which the corrected cancer rate is being calculated.

Σ denotes summation over all ages s.

This calculation is more satisfactory than the method of simple percentages since (a) it takes into consideration the changing incidence of tumor as age advances. (b) It gives all of the strains an equal opportunity to have cancer, at the various age periods.

Table VII illustrates the computation of corrected cancer rates when the sum of 3 breeding populations

TABLE II: REFINEMENT OF METHOD OF SIMPLE RATIOS

Stock	No. in experiment	No. with cancer	Percentage of cancer
Dilute brown	3,165	2,091	66.1
Bittner albino	713	526	73.8
Marsh albino	1,341	1,031	76.9

TABLE III: PERCENTAGES OF MAMMARY CANCER AMONG MICE LIVING AT THE BEGINNING OF SUCCESSIVE CHRONOLOGICAL AGE PERIODS

Months	Dilute brown	Marsh albino
1	64.5	76.4
2	64.8	76.4
3	66.1	76.5
4	67.5	76.7
5	69.8	76.9
6	72.4	77.4
7	75.4	77.8
8	78.9	77.7
9	82.4	78.0
10	86.0	78.0
11	86.5	77.4
12	85.2	76.3
13	84.0	76.0
14	85.2	74.2
15	84.9	72.3
16	84.5	68.7
17	79.3	66.3
18	77.2	63.6
19	75.0	60.3
20	83.3	60.0
21	100.0	59.5
22	....	57.9
23	....	33.3

(dilute brown, Bittner albino, and Marsh albino) is used as a standard population.

This table may be summarized as follows:

	Dilute brown	Bittner albino	Marsh albino
Corrected cancer rates per 10,000 population	957	707	380
Ratio of rates	100	73.9	39.7

It is evident from these figures that the relationship of these 3 stocks, when compared by this method, is the reverse of that shown by the summary of the method of simple percentages. The ratio between the Bittner albino and Marsh albino stocks, when computed by this method, is in striking contrast to their relationship when computed by the method of percentages.

TABLE IV: OBSERVED NUMBERS OF MICE AT THE DIFFERENT AGE INTERVALS, WITH CALCULATED PER CENT OF SURVIVORS AND PER CENT OF LIFE SPAN

	Breeding females								
	Dilute brown			Bittner albino			Marsh albino		
Age interval 30 days	No. alive at beginning of age interval	Per cent of survivors at beginning of age interval	Age in per cent of life span (Life span = 18 months)	No. alive at beginning of age interval	Per cent of survivors at beginning of age interval	Age in per cent of life span (Life span = 20 months	No. alive at beginning of age interval	Per cent of survivors at beginning of age interval	Age in per cent of life span (Life span = 22 months)
1-2	3,241	100	5.5	730	100	5	1,350	100	4.5
2-3	3,226	99.5	11.1	725	99.3	10	1,350	100	9.1
3	3,165	97.6	16.5	719	98.5	15	1,348	99.9	13.6
4	3,090	95.3	22.2	713	97.7	20	1,348	99.9	18.1
5	2,975	91.8	27.8	684	93.7	25	1,341	99.3	22.7
6	2,803	86.5	33.3	664	91.0	30	1,331	98.6	27.2
7	2,560	79.0	38.9	636	87.1	35	1,308	96.9	31.8
8	2,211	68.2	44.4	596	81.6	40	1,265	93.7	36.3
9	1,804	55.7	50.0	542	74.2	45	1,199	88.8	40.8
10	1,396	43.1	55.5	460	63.0	50	1,117	82.7	45.4
11	980	30.2	61.0	378	51.8	55	1,010	74.8	50.0
12	628	19.4	66.7	307	42.1	60	891	66.0	54.5
13	404	12.5	72.3	227	31.1	65	780	57.8	59.0
14	250	7.7	77.7	157	21.5	70	651	48.2	63.6
15	172	5.3	83.3	101	13.8	75	516	38.2	68.6
16	103	3.2	88.8	62	8.5	80	379	28.1	72.7
17	58	1.8	94.5	35	4.8	85	285	21.1	77.3
18	35	1.08	100.0	26	3.6	90	206	15.3	81.8
19	16	0.49	...	14	1.9	95	141	10.4	86.3
20	6	0.18	...	7	0.96	100	85	6.3	90.8
21	3	0.09	...	3	0.4	...	42	3.1	95.3
22	...	...	...	2	0.3	...	19	1.4	100.0
23	...	...	...	1	0.1	...	3	0.2	...

TABLE V: OBSERVED NUMBERS OF MAMMARY TUMORS AT THE DIFFERENT AGE INTERVALS AND CALCULATED RATES OF TUMOR INCIDENCE FROM THE SURVIVAL NUMBERS OF TABLE IV

	Breeding females					
	Dilute brown		Bittner albino		Marsh albino	
Age interval in months (30 days)	Mammary cancer found in age interval	Rate of incidence of cancer per 10,000	Mammary cancer found in age interval	Rate of incidence of cancer per 10,000	Mammary cancer found in age interval	Rate of incidence of cancer per 10,000
1-2	...	...	..	...	...	...
2-3	...	...	..	...	...	...
3	4	12	..	...	...	...
4	11	35	2	28	...	...
5	36	122	3	43	1	7
6	111	396	9	105	13	97
7	184	718	23	361	34	259
8	258	1,166	34	570	48	379
9	297	1,646	71	1,310	65	542
10	342	2,449	65	1,413	88	787
11	313	3,193	60	1,587	102	1,010
12	194	3,089	67	2,149	88	987
13	128	3,168	62	2,731	109	1,397
14	67	2,680	45	2,866	110	1,689
15	59	3,430	30	2,970	113	2,190
16	41	3,980	25	4,016	71	1,873
17	19	3,275	8	2,285	58	2,035
18	15	4,285	11	4,231	46	2,233
19	7	4,500	4	2,857	34	2,411
20	2	3,333	4	5,714	26	3,058
21	3	10,000	1	3,333	14	3,333
22	...	...	1	5,000	10	5,263
23	...	...	1	10,000	1	3,333
	2,091		526		1,031	

The Choice of a Standard Population

The use of the method of corrected cancer rates brings out the problem of the proper choice of a standard population. While the standard population, here used, seems to have approximately the same life span as the 3 breeding female populations, it is considerably shorter than that of the virgin BdF1 population, a cross obtained by mating C57 black females with dilute brown males (Table VII). An attempt to compute the corrected cancer rate for the BdF1 generation, in order to compare it with the breeding female populations, will produce misleading results. It is seen that the standard population has died out by the time the BdF1 generation begins to have tumors in appreciable numbers.

While the example of the BdF1 generation in comparison with the standard population of breeding females is an extreme case of the disparity between a population and a given standard, it points to a fundamental problem in the computation of cancer rates. It suggests that even though the 3 breeding female populations have approximately the same life span, they should not be compared with such a standard at the same chronological ages.

Table VI: Observed Numbers at the Different Age Intervals, Calculated Per Cent of Survivors, Calculated Percentages of Life Span

	Virgin females								
	V_1			V_2			V_3		
Age in months (30 days)	Number alive at beginning of age interval	Per cent of survivors at beginning of age interval	Age in per cent of life span (Life span = 35 months)	Number alive at beginning of age interval	Per cent of survivors at beginning of age interval	Age in per cent of life span (Life span = 30 months)	Number alive at beginning of age interval	Per cent of survivors at beginning of age interval	Age in per cent of life span (Life span = 26 months)
1-2	3,136	100	2.86	695	100	3.53	397	100	3.8
2-3	3,136	100	5.72	695	100	6.66	397	100	7.7
3	3,136	100	8.58	695	100	9.99	397	100	11.5
4	3,136	100	11.4	695	100	13.4	397	100	15.4
5	3,136	100	14.3	688	99	16.6	396	99.7	19.2
6	3,129	99.9	17.1	680	97.8	20.0	394	99.3	23.1
7	3,124	99.7	20.0	678	97.4	23.3	393	99.0	26.9
8	3,113	99.4	22.9	672	96.7	26.6	391	98.5	30.7
9	3,105	99.1	25.7	671	96.6	29.9	389	98.0	34.5
10	3,081	98.3	28.6	667	96.0	33.3	385	97.0	38.4
11	2,992	95.5	31.5	664	95.5	36.6	380	95.7	42.2
12	2,863	91.3	34.3	661	95.1	39.9	367	92.4	46.2
13	2,698	86.0	37.2	657	94.5	43.3	345	87.0	50.0
14	2,530	80.7	40.0	655	94.3	46.6	322	81.1	43.8
15	2,369	75.5	42.8	649	93.4	50.0	292	73.6	57.6
16	2,241	71.5	45.7	637	91.7	53.3	254	64.0	61.4
17	2,121	67.7	48.7	628	90.4	56.6	206	51.9	65.4
18	2,019	64.4	51.4	617	88.8	59.9	163	41.0	69.3
19	1,901	60.7	54.3	603	86.8	63.3	128	32.2	73.1
20	1,788	57.1	57.2	559	80.5	66.6	94	23.7	77.0
21	1,668	53.2	60.0	516	74.3	69.9	61	15.4	80.8
22	1,536	49.0	62.9	477	68.7	73.3	35	8.8	84.6
23	1,409	45.0	65.8	408	58.7	76.6	20	5.0	88.5
24	1,287	41.1	68.7	340	49.0	79.9	13	3.1	92.3
25	1,131	36.1	71.5	290	41.7	83.3	...	...	96.1
26	990	31.6	74.3	209	30.1	86.6	...	...	100
27	875	27.9	77.2	127	18.2	89.9	...	...	...
28	727	23.1	80.0	49	7.0	93.3	...	...	...
29	581	18.5	82.8	13	1.8	96.6	...	...	...
30	441	14.0	85.8	2	0.2	100	...	...	...
31	301	9.6	88.6	...	...	...	...	...	...
32	178	5.7	91.5	...	...	...	...	...	...
33	88	2.8	94.3	...	...	...	...	...	...
34	66	2.1	97.2	...	...	...	...	...	...
35	31	0.99	100	...	...	...	...	...	...
36	21	0.67	...	...	...	...	...	...	...
37	6	...	...	...	...	...	...	...	...
38	2	...	...	...	...	...	...	...	...
39	1	...	...	...	...	...	...	...	...

TABLE VII: CALCULATION OF CORRECTED CANCER RATES USING A STANDARD POPULATION WHICH IS THE SUM OF THE 3 BREEDING FEMALE POPULATIONS. CANCER RATES HERE ARE TAKEN FROM SMOOTHED GRAPHS OF OBSERVED DATA (FIG. 3)

Age interval in months	Per cent of survivors of breeding females—Dbr, Marsh albino, Bittner albino	Marsh albino		Bittner albino		Dilute brown		BdF1	
		Cancer rates from Fig. 3	Calculated cancers from standard population	Cancer rates from Fig. 3	Calculated cancers from standard population	Cancer rates from Fig. 3	Calculated cancers from standard population	Observed rates of cancer incidence	Calculated cancers from standard population
1-2	100	...	...	...	...	...	...	...	..
2-3	99.6	...	...	...	...	...	...	...	..
3	98.3	...	...	9	9	11	11	...	..
4	96.6	...	...	20	19	34	33	...	..
5	94.0	...	...	45	42	123	116	...	..
6	90.0	90	81	116	104	330	297	...	..
7	84.6	250	212	330	279	740	626	6.5	5
8	76.5	392	300	780	597	1,240	949	13.8	10
9	66.6	560	373	1,250	832	1,830	1,219	19.5	13
10	58.8	770	453	1,660	976	2,350	1,382	24.5	13
11	44.5	1,000	445	2,010	894	2,740	1,219	28	12
12	34.3	1,250	429	2,300	789	2,980	1,022	30	10
13	26.5	1,500	398	2,600	689	3,120	827	32	8
14	19.8	1,710	339	2,770	548	3,280	649	34	7
15	14.8	1,850	274	3,100	459	3,390	502	36	5
16	10.2	1,980	202	3,370	344	3,460	353	39	4
17	7.1	2,130	151	3,660	260	3,560	253	42	3
18	5.0	2,260	113	3,950	189	3,700	185	45	2
19	3.2	2,470	79	4,300	138	3,860	124	49	2
20	1.8	2,720	49	4,680	84	4,100	74	53	1
21	0.9	3,080	28	4,900	44	4,500	41	57	1
22	1,033.1		3,926		7,305		9,882	61	96
23								67	
24								73	
25	Corrected rate per 10,000.....		380		707		957	80	9.3
26								89	
27	Ratio of rates.....		39.7		73.9		100	100	0.97
28								115	
29								133	
30								160	
31								195	
32								245	
33								310	
34								390	
35								500	

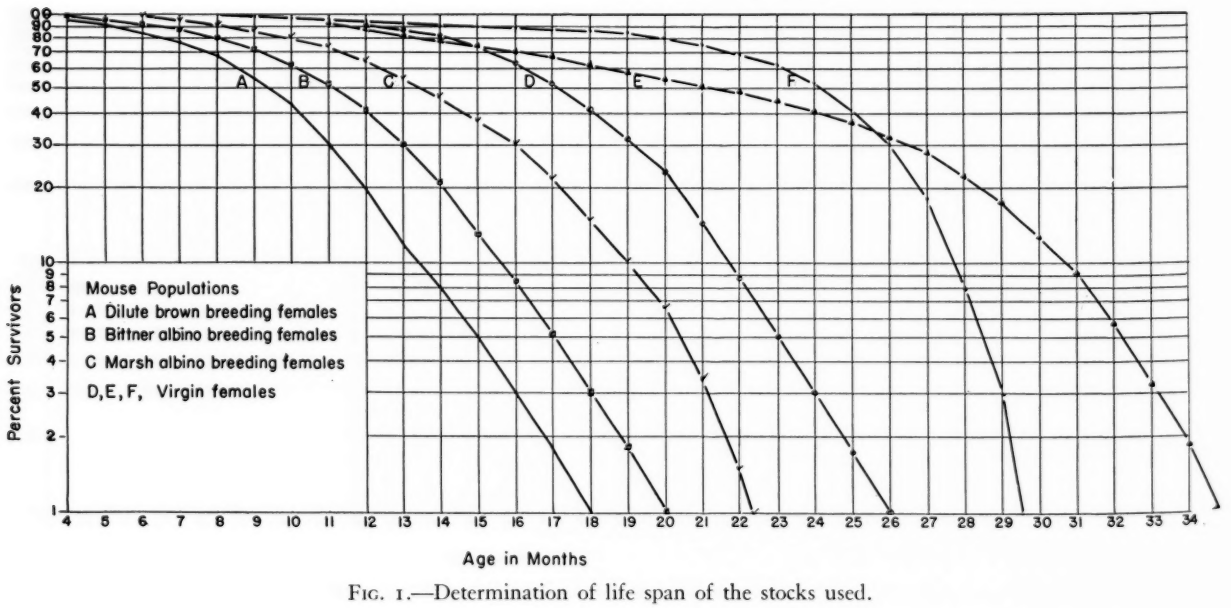


FIG. 1.—Determination of life span of the stocks used.

Physiological Standardized Population

The difficulties arising from differences in life span of strains of mice are avoided in the proposed method, which is based on physiological age.[3]

When the data of Tables IV and VI are plotted on an arithlog grid, the curves of Fig. 1 are obtained. The intercepts of these curves on the time axis, at 1 per cent of survivors, give the life spans of the various populations of mice. Once the life span is determined, it is possible to convert the various chronological ages to centiles of life span by use of the formula $C = 100\ (a/b)$,[4] and to plot the numbers thus obtained against the percentages of survivors (Table IV).

The survival values of the breeding females of 3 strains and of virgin mice of 2 strains at the various centiles of their physiological life spans are shown in Fig. 2.

An obvious standard population may be constructed by combining the 3 stocks of breeding females, in the following manner: (a) The 3 stocks are plotted as shown in Fig. 2. (b) The average per cent of survivors of the 3 stocks is taken at intervals of 5 centiles of life span. The resultant population, which may be called the physiologically standardized population of breeding females, is shown in Fig. 4. On this graph, a physiologically standardized population which is the average of the 3 strains of virgin mice[5] given in Table VI, is also shown.

The curves on this figure indicate that the virgin mice have a distinctly lower mortality. At 75 centiles of life span, 42 per cent of the virgins are still alive compared with 16.5 per cent of the breeding females.

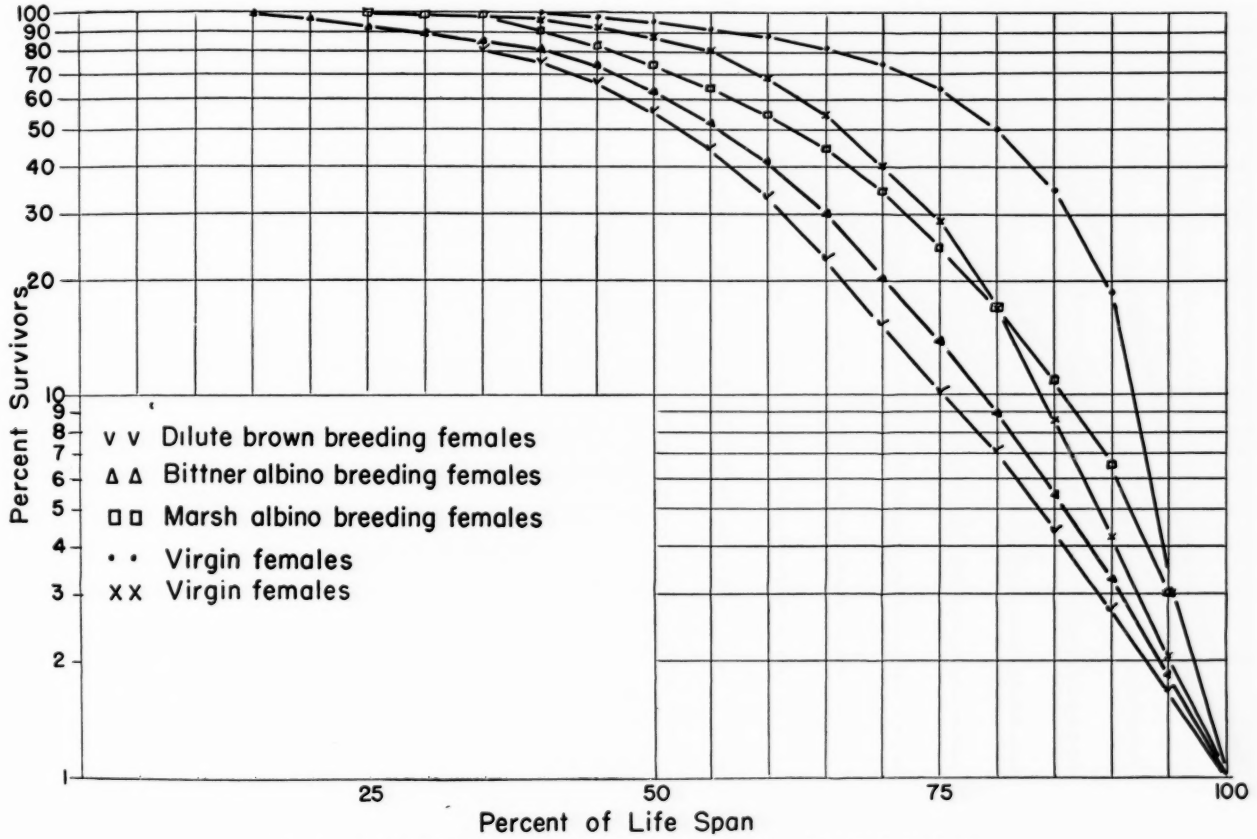


Fig. 2.—Survival values of strains of mice in centiles of physiological age.

Derivation of Cancer Rates at the Various Centiles of Life Span

In order to get cancer rates at the various physiological ages, columns 3, 5, and 7 of Table V are plotted against columns 4, 7, and 10 of Table IV, upon the arithlog grid. Smoothed curves are then drawn

[3] Physiological age is here measured in terms of the life span or the ability of the various strains to survive. For the sake of convenience, life span is here defined arbitrarily as the period of time between birth and that age at which only 1 per cent of a population survives. The figure, 1 per cent, is chosen because it is convenient for practical graphical purposes. With sufficient data, 0.1 per cent might be chosen as the limiting fraction of survivors (7a).

[4] In this formula, C equals chronological age expressed in hundredths of life span; b is the age at which 1 per cent of the population survives; and a is any chronological age.

[5] Derivation of virgin populations used in Table VI: V_1 and V_2 are taken from Tables III and XVI published by Murray and Little in 1939 (6). V_3 are from previously unpublished data concerning virgin Marsh albino females.

through the observed cancer rates for each stock. The rates of incidence for the 3 breeding female populations, when plotted in this manner, are illustrated in Fig. 3. These curves give the complete picture of the incidence of tumors in these stocks throughout their life spans. It will be noted that the rate of cancer incidence approaches a constant value toward the end of the life span.

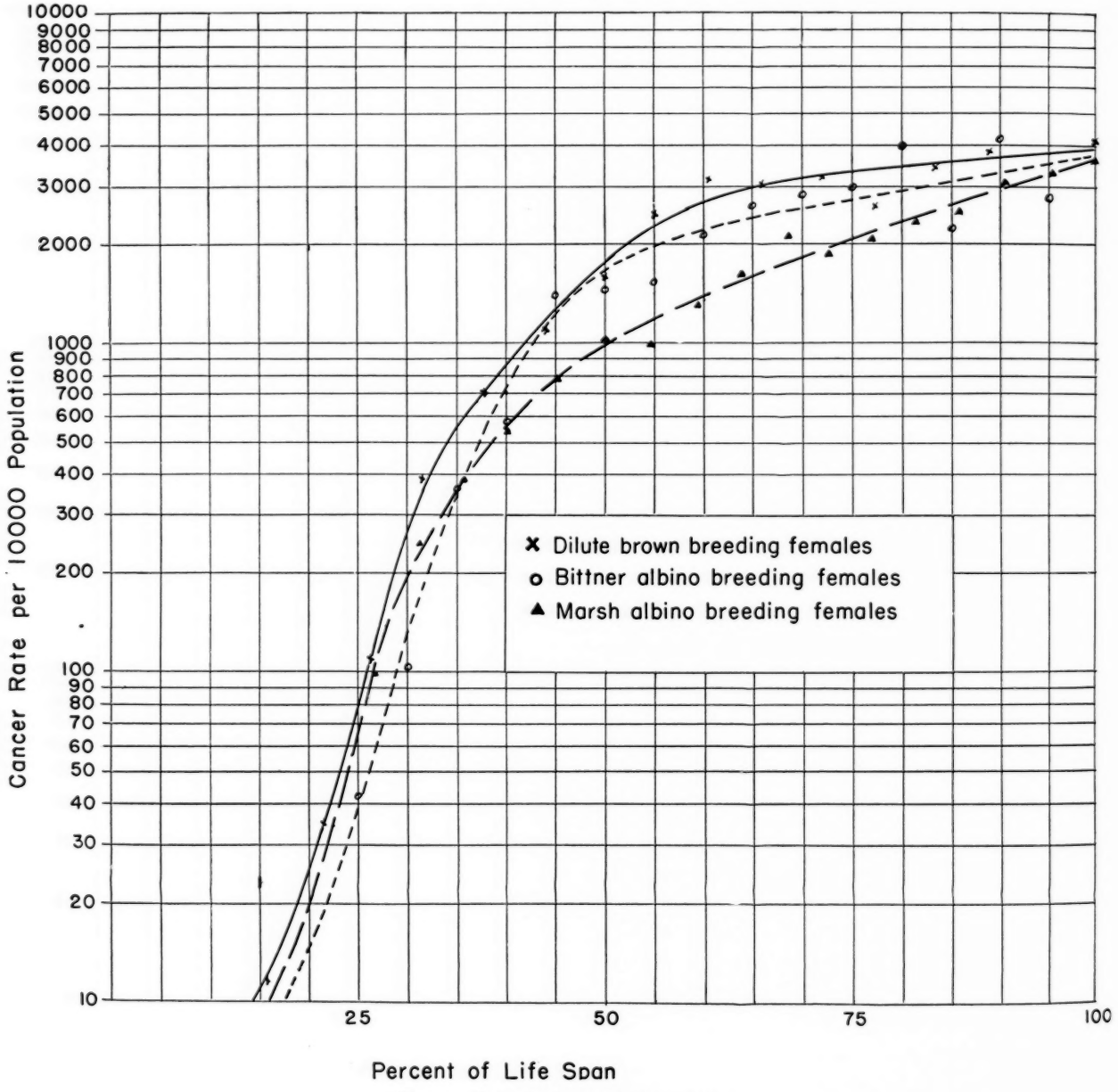


FIG. 3.—Smoothed cancer rate curves.

METHODS WHICH DISPENSE WITH STANDARD POPULATION

In order to dispense with a standard population, use may be made of an assumed population in which there are equal percentages of survivors at each physiological age. This would be a kind of "robot" population in that all individuals would live to exactly the same age and then die instantly. This method may be illustrated as follows: The cancer incidence rates for the breeding females in Table VIII are summed and give indices which may be used for comparison. The amounts of cancer in the dilute brown, Bittner albino, and Marsh albino strains when computed in this manner are to each other as 100:86.1:69.8, respectively. This method is subject to the objection that it tends to reduce the sensitivity of a comparison because it contributes large numbers of cancers at the old age periods where the cancer rates are at a maximum and the per cent of survivors in any living population is tending toward zero.

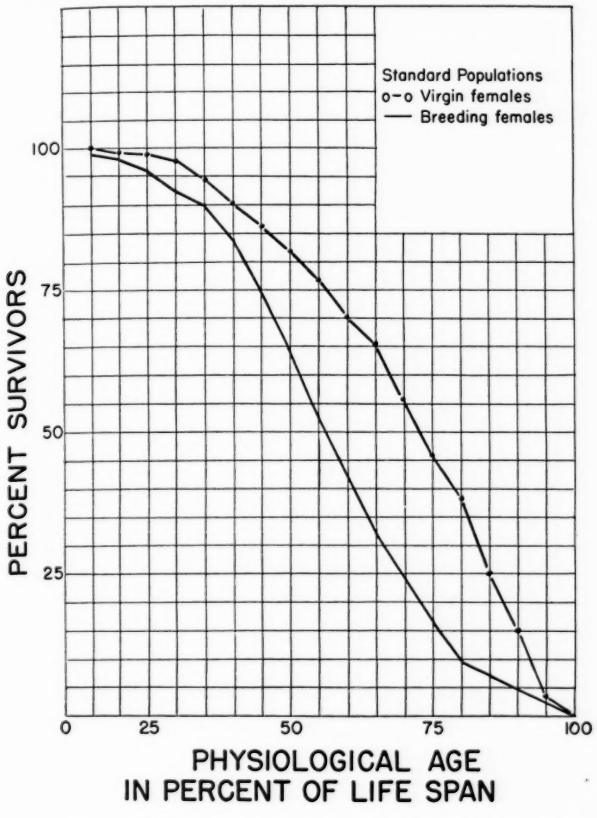


FIG. 4.—Mortality curves of standard population.

TABLE VIII: CALCULATION OF CORRECTED CANCER RATES USING A "ROBOT" POPULATION. CANCER RATES TAKEN FROM SMOOTHED CURVES (FIG. 3)

	Breeding females		
Centiles of life span	Dilute brown Cancer incidence rates	Marsh albino Cancer incidence rates	Bittner albino Cancer incidence rates
5	...	...	...
10	...	...	...
15	11	...	...
20	24	20	15
25	75	70	42
30	305	200	133
35	600	360	340
40	920	550	650
45	1,250	770	1,190
50	1,660	1,000	1,600
55	2,250	1,210	1,950
60	2,850	1,450	2,250
65	3,150	1,650	2,500
70	3,300	1,880	2,700
75	3,400	2,100	2,850
80	3,450	2,350	2,980
85	3,520	2,610	3,100
90	3,600	2,920	3,300
95	3,700	3,300	3,450
100	3,850	3,700	3,600
	37,915	26,140	32,650
Corrected rates	1,896	1,307	1,633
Ratio	100	69	86

(Corrected rates are derived by dividing the sum of incidence rates by the number of variates.)

TABLE IX: CALCULATION OF CORRECTED CANCER RATES ON A PHYSIOLOGICAL BASIS USING THE VIRGIN FEMALE STANDARD POPULATION. CANCER RATES OF INCIDENCE THE SAME AS IN TABLES VIII AND X

		Breeding females					
		Dilute brown		Marsh albino		Bittner albino	
Centiles of life span	Per cent of survivors standard population, virgin mice	Cancer rates per 10,000	Expected cancer	Cancer rates per 10,000	Expected cancer	Cancer rates per 10,000	Expected cancer
5	100	...	...	...	...	...	...
10	100	...	...	...	...	...	...
15	100	11	11	...	...	...	...
20	99	24	24	20	20	15	15
25	99	75	74	70	69	42	42
30	98	305	299	200	196	133	130
35	94.4	600	566	360	340	340	321
40	90.6	920	834	550	498	650	589
45	89.3	1,250	1,163	770	687	1,190	1,063
50	82	1,660	1,361	1,000	820	1,600	1,312
55	76.6	2,250	1,724	1,210	927	1,950	1,494
60	69.8	2,850	1,989	1,450	1,012	2,250	1,571
65	60.8	3,150	1,915	1,650	1,003	2,500	1,520
70	51.0	3,300	1,683	1,880	959	2,700	1,377
75	41.3	3,400	1,404	2,100	867	2,850	1,177
80	30.8	3,450	1,063	2,350	724	2,980	918
85	20.0	3,520	704	2,600	520	3,100	620
90	9.7	3,600	349	2,920	283	3,300	320
95	2.8	3,700	104	3,300	92	3,450	97
100	1.0	3,850	39	3,700	37	3,600	36
	1,316.1		15,306		9,054		12,602
Corrected cancer rates per 10,000			1,163		688		958
Ratios			100		59.2		82.4

TABLE X: CALCULATION OF "ABSOLUTE" AMOUNT OF CANCER FROM OBSERVED RATES OF MAMMARY TUMORS AND OBSERVED SURVIVAL RATIOS. ALSO A CALCULATION OF CORRECTED CANCER RATE FOR EACH STRAIN

	Breeding females								
	Dilute brown			Marsh albino			Bittner albino		
Centiles of life span	Cancer incidence rates	Per cent of survivors at physiological ages	Cancers at physiological ages	Cancer incidence rates	Per cent of survivors at physiological ages	Cancers at physiological ages	Cancer incidence rates	Per cent of survivors at physiological ages	Cancers at physiological ages
5	...	100	...	...	100	...	...	100	...
10	...	99	...	...	100	...	...	100	...
15	11	98	11	...	100	...	...	99	...
20	24	96	23	20	100	20	15	98	15
25	75	93	70	70	100	70	42	96	40
30	305	89	271	200	99	198	133	92	122
35	600	85	510	360	96	346	340	88	299
40	920	78	718	550	90	495	650	82	533
45	1,250	68	850	770	83	639	1,190	73	869
50	1,660	55.5	921	1,000	74	740	1,600	63	1,008
55	2,250	45	1,013	1,210	65	787	1,950	52	1,014
60	2,850	32	912	1,450	55	798	2,250	42	945
65	3,150	22.5	709	1,650	44	726	2,500	31	775
70	3,300	15.8	521	1,880	34	639	2,700	21.5	581
75	3,400	10.5	357	2,100	24.5	515	2,850	14.5	413
80	3,450	6.9	238	2,350	17.5	411	2,980	9.3	277
85	3,520	4.4	155	2,600	11.2	291	3,100	5.7	177
90	3,600	2.8	100	2,920	6.6	193	3,300	3.4	112
95	3,700	1.7	63	3,300	3.1	102	3,450	1.9	66
100	3,850	1.0	39	3,700	1.0	37	3,600	1.0	36
	37,915		7,481	26,130		7,007	32,650		7,105
Per cent cancer calculated			74.8			70.0			71.0
Ratios of calculated cancer			100			93.5			94.9
Corrected cancer rates per 10,000			745			582			662
Ratio of corrected rates			100			78.1			88.9

A second method, which dispenses with a standard population, is that in which the amount of cancer is calculated from the smoothed data of cancer rates and the observed per cent of survivors in the strain. This system of computation is illustrated by Table X, which lists the number of cancers which may be expected at each centile of life span in the 3 breeding populations. Strictly speaking, these corrected cancer rates are not comparable, since each has a different population distribution. The amounts of cancer are, however, comparable. The amounts of cancer calculated in this manner may be termed the "absolute" amounts, since this method makes use of the crude data without resort to a fictitious standardizing method.

DISCUSSION

Table XI summarizes the results obtained from the same data when different types of calculations, which have been described, are used. It shows that a wide variety of figures and relationships are obtainable, depending upon the method used.

Simple percentage.—The method of simple percentage of cancer, while easily applied, gives a limited

TABLE XI: COMPARISON OF METHODS OF COMPUTING AMOUNTS OF CANCER

	Breeding females		
	Dilute brown	Bittner albino	Marsh albino
Observed percentages of mammary cancer. (Table I.)	64.5	72.0	76.4
Ratio of percentages	100	112	118
Calculation of "absolute" amount of cancer. (Table X.)	74.8	71.0	70.0
Ratio of percentages	100	94.9	93.5
Robot standard population, corrected cancer rates per 10,000. (Table VIII.)	1,896	1,633	1,307
Ratio of rates	100	86	69
Corrected cancer rates per 10,000 using chronologically standard population of breeding females. (Table VII.)	957	707	380
Ratio of rates	100	73.9	39.7
Corrected cancer rates per 10,000 using virgin female physiological standard population. (Table IX.)	1,163	958	688
Ratio of rates	100	82.4	59.2

amount of information since it does not take into account the variation of the physiological well-being among the different strains.

Absolute amount.—The method of calculating the absolute amount of cancer does not take into consideration the physiological variation among strains. It uses the data directly, in that it makes use of the rates of cancer incidence over the entire life span. It is a logical refinement of the method of simple percentages. If simple percentages are to be used as a basis for the comparison of strains, they should be calculated by the method of absolute amount. This method has one serious disadvantage. It does not give correct rates of cancer incidence for the different strains which are comparable. While the calculations used here are carried out on the basis of physiological age, they might just as well have been obtained upon a chronological age basis.

Robot population.—The arbitrariness of the choice of a standard population is avoided in the robot population; that is, an assumed population in which there are the same number of survivors at all physiological ages. This type of population is universally applicable and has been advocated by statisticians in preference to standard populations, as brought out in the discussion at the end of Yule's paper (10).

Chronological age versus physiological age.—The calculation shown in Table VII, on a chronological basis gave unreliable results because of the possible disparity in life spans among the populations to be measured. Even though the standard population used here has approximately the same life span as the strains which are to be measured by it, there is no sound theoretical basis for carrying out the calculation. This becomes especially important when measuring cancer rates, which are a decided function of physiological age.

It seems then, that the method of comparing strains on a physiological basis with an appropriate standard population meets the objections encountered in the other methods. The physiologically standardized population of virgin females is here chosen as the standard with which the breeding females shall be compared. The calculations shown in Table IX give the amounts of cancer which might have been expected in the standard virgin females had they been subjected to the physiological burdens which the breeding females bear. In accordance with recognized statistical theory this method gives corrected cancer rates which are comparable.

The use of a physiologically standardized population of virgin females may be objectionable on the ground that virgin females in some strains are subject to an appreciable number of spontaneous tumors, a fact which affects their physiological well-being. If the virgin females have appreciably large amounts of cancer, resort may be made to a male population or perhaps to a virgin female population of some other strain which has a small incidence of cancer.

Summary

1. The various prevalent methods of comparing the amounts of spontaneously occurring mammary cancer in different strains of mice are applied to 3 populations of breeding females (dilute brown, Bittner albino, and Marsh albino).

2. A method of calculating corrected cancer rates on a physiological basis is proposed. This method takes into account the physiological variability among strains of mice as well as the strong dependence of cancer incidence upon physiological age.

3. The cancer incidence which would have occurred in virgin female mice, had they had the same rates of mammary cancer incidence as the breeding females, indicates that the breeding females of the dilute brown, Bittner albino, and Marsh albino strains have cancer rates which are to each other as 100:82:59, respectively.

REFERENCES

1. Bittner, J. J., and W. S. Murray. Comparative Study of Four High Tumor Lines of Mice. Am. Nat., **70**:443-453. 1936.
2. Korteweg, R. Heredity of Cancer of the Mammary Gland in Mice. Program of Third International Cancer Congress, September 11, 1939. p. 85.
3. Murray, W. S. The Breeding Behavior of the Dilute Brown Stock of Mice (Little dba). Am. J. Cancer, **20**:573-593. 1934.
4. Murray, W. S. Genetic Segregation Mammary Cancer to No Mammary Cancer, in the Mouse. Am. J. Cancer, **34**:434-441. 1938.
5. Murray, W. S. Physiological Age Compared with Chronological Age as a Means of Computing Mammary Cancer Rates in Mice. Program of Third International Cancer Congress, September 11, 1939. pp. 85-86.
6. Murray, W. S., and C. C. Little. Chromosomal and Extrachromosomal Influence in Relation to the Incidence of Mammary Tumors in Mice. Am. J. Cancer, **37**:536-552. 1939.
7. Pearl, R. Medical Biometry and Statistics. W. B. Saunders Co., Philadelphia, 1923. (a) p. 191. (b) pp. 202-208.
8. Rahn, O. Chemistry of Death. Cold Spring Harbor Symposia on Quantitative Biology, **2**:70-77. 1934.
9. Rahn, O. The Size of Bacteria as the Cause of the Logarithmic Order of Death. J. Gen. Physiol., **13**:179-205. 1929.
10. Yule, U. On Some Points Relating to Vital Statistics, More Especially Statistics of Occupational Mortality. J. Roy. Statist. Soc., **97**:1-72. 1934.

Failure to Find Carcinogens in Urine from Patients with Cancer

Russel O. Bowman, Ph.D., and H. R. Mottshaw, A.M.

(From the Rhode Island Hospital Laboratory and Brown University, Providence, Rhode Island)

(Received for publication March 3, 1941)

In 1939 we began a series of experiments designed to detect a possible cancer-producing substance in the urine of individuals affected with cancer. Researches of others up to that time had indicated that if such an agent existed and were excreted in the urine it might be in one of three possible forms: (a) a heavy-molecular protein, analogous to Stanley's tobacco mosaic virus (2), (b) a growth-stimulating factor, already separated by Rohdenburg and Nagy (1), or (c) a chemical compound related to the experimental carcinogenic derivatives of coal tar, which would also be similar to cholesterol, the bile acids, the sex hormones, and other known body constituents.

Five 2000-cc. samples of urine collected from patients known to have cancer were treated by a salting-out procedure described by Stanley for use with macerated plant tissues (2). No end product was obtained in any case, although a qualitative test for protein was positive in three of the cases. Even if a carcinogen were present in the tissues as a protein of high molecular weight it probably would not be excreted, since such a molecule could not pass through the membranes of the cell or the intact glomerulus of the kidney.

TABLE I: MICE INJECTED WITH GROWTH-STIMULATING AND INHIBITING SUBSTANCES

	Stimulator	Inhibitor
Total number of mice injected	20	5
Injected only once before death	11	1
Injected for 8 to 20 weeks before death	5	0
Injected for 32 to 40 weeks	4	4

By following the procedure of Rohdenburg and Nagy (1) growth-stimulating and growth-inhibiting fractions were separated from twenty 1000-cc. samples of urine from patients with cancer. Four samples of stimulator and 3 of inhibitor were used. The preparations were not assayed on *Colpidium campylum* but were injected into mice. We used only adult mice, carefully inbred from parents of the C57 black strain obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. They were injected once a week, subcutaneously in the groin, with 0.1 to 0.5 cc. of a 50 per cent saline suspension of the dried stimulator or inhibitor. The results of this experiment are summarized in Table I. Eight mice lived for more than 280 days after the start of injections, 4 of them for more than 500 days before they were killed. Postmortem examinations and sections of organs revealed no tumor growth in any of the animals. The 5 mice injected with the inhibitor preparation were used as controls.

TABLE II: MICE TREATED WITH URINE EXTRACTS

Benzene extract of urine	Ether extract of urine
TARRING	
Five mice had 17 weekly applications, lived an additional 250 days before sacrifice	Three mice had 17 weekly applications, lived an additional 250 days before sacrifice
SINGLE INJECTION IN OLIVE OIL	
0.2 and 0.4 cc. One mouse for each, died in less than 90 days	0.2 and 0.4 cc. One mouse for each, died in less than 90 days
SINGLE INJECTION IN SALINE SUSPENSION	
0.4 cc. Five mice, 2 of which died in 24 hours; 3 lived for 100 days, were then sacrificed	0.4 cc. Five mice, 1 of which died in 24 hours; 4 lived for 100 days, were then sacrificed

Two batches of 200 liters of pooled urine from patients with cancer were collected at the Rhode Island Hospital and St. Elizabeth's Hospital in Providence. These were evaporated at 90 to 98° C. to a semisolid state and extracted in the evaporating beakers with 6 portions of thiophene-free benzene and then with 6 portions of ethyl ether. The benzene and the ether extracts collected for each batch amounted to about 50 liters. After evaporation of the solvent, black oily gums weighing 4.5 and 1.5 gm. were obtained from the benzene extracts, and similar materials weighing 15.4 and 3.2 gm. were obtained from the ether extracts. These residues were diluted to 100 cc. each with their respective solvents and used for tarring experiments. Aliquots were evaporated to dryness and dissolved in the same volumes of saline solution or of olive oil for single injections into the groin of mice.

The olive oil solutions in amounts of 0.2 and 0.4 cc., equivalent to 400 and 800 cc. of urine, were found to be quite toxic, and the longest survival was 3 months for a mouse injected with 0.2 cc. of a benzene extract.

Saline suspensions were less toxic. Some mice could be injected with the equivalent of 800 cc. of urine and live (Table II). No changes indicative of tumor formation were found, however, when the mice were sacrificed and their organs sectioned.

In conclusion, with the above experimental methods we were unable to demonstrate any substance present in the urine of patients with cancer which would produce tumors in C57 black mice.

REFERENCES

1. Rohdenburg, G. L., and S. M. Nagy. Growth Stimulating and Inhibiting Substances in Human Urine. Am. J. Cancer, **29**:67-77. 1937.
2. Stanley, W. M. Chemical Studies of the Virus of Tobacco Mosaic. VII. An Improved Method for the Preparation of Crystalline Tobacco Mosaic Virus. J. Biol. Chem., **115**:673-678. 1936.

The Effect of Lowered Atmospheric Pressure on Spontaneous Tumors in Mice.

G. L. Rohdenburg, M.D.

(From the Achelis Laboratory, Lenox Hill Hospital, New York, N. Y.)

(Received for publication March 8, 1941)

In February, 1940, Dr. A. Hodes of Holland, in a personal communication, presented the idea outlined in a succeeding paragraph, and stated that following this technic he had been able to induce recession of spontaneous malignant tumors in mice. He stated that he would forward his publications, but these have not arrived, and we have not been able to locate any of them in the usual collections; such as, the *Quarterly Cumulative Index Medicus*. For these reasons, no bibliographic references can be given.

According to Hodes, if mice bearing spontaneous tumors are kept at a temperature of 36° + C. in a chamber containing 10 per cent carbon dioxide, 34 per cent nitrogen, and 56 per cent oxygen, and the pressure of these combined gases is reduced to 38 cm. of Hg, the tumors disappear after a period of from 4 to 6 weeks. We shall not present the theoretical basis for this particular combination advanced by Hodes.

On the basis of the description given by Hodes, a chamber of 5 gallons capacity was so constructed as to enable one to keep the temperature desired, to mix the gases in the required percentages, and to keep the pressure within 1 cm. of the desired point.

In the first series of observations, 36 mice, in groups of 6 animals each, were inoculated with mouse sarcoma 180. On the sixth post-inoculation day, the mice were placed in the chamber. It was at once noted that the animals refused food unless the pressure was brought back to about 50 cm. Hg and that in consequence of not eating, they became emaciated and finally died in from 7 to 20 days after the onset of the experiment. The animals duplicated the experiences of some human beings in the last World War, for despite imposed starvation the tumors grew. Statements which are so often made to the effect that the rate of growth is slower than normal (whatever normal may be) are based on such unreliable criteria that they lack all value. Post-mortem histological examination of the tumors showed no changes not ordinarily observed in animals not subjected to any form of treatment.

The experiment was repeated with 12 mice bearing spontaneous carcinomas of the breast (Roscoe B. Jackson Memorial Laboratory, A, C3H, Dba strains). The results observed up to 21 days were the same as those previously described. All of the animals died of starvation; no tumors disappeared.

Conclusions

Lowering the atmospheric pressure, raising the temperature to 36° C. and placing the tumor-bearing mice in an atmosphere containing 10 per cent carbon dioxide, 34 per cent nitrogen, and 56 per cent oxygen for periods up to 3 weeks did not cause either transplanted mouse sarcoma 180 or spontaneous mouse carcinoma to disappear.

The author wishes to express his appreciation to Dr. W. H. Woglom of the Department of Cancer Research of Columbia University for supplying the mice inoculated with mouse sarcoma 180.

The Glucose Tolerance Test in Its Relation to Cancer*

G. L. Rohdenburg, M.D.

(From the Achelis Laboratory, Lenox Hill Hospital, New York, N. Y.)

(Received for publication March 8, 1941)

D. Jackson and D. A. Todd (2) have presented the idea that a study of the carbohydrate metabolism in malignant tumor bearers would be of aid in determining the degree of malignancy of the tumor. In their conclusions they state: "In the presence of malignancy, carbohydrate tolerance is definitely decreased, the degree of decrease varying directly with the degree of malignancy of the tumor." If this could be definitely established, it would prove to be of great aid to the clinician. In 1934 it was determined to test the validity of the idea on the material available in the wards of Lenox Hill Hospital, the group participating in the investigation being the writer, Dr. A. Bernhard, Dr. Carl Eggers, and Dr. P. K. Sauer.

In accordance with the routine described by Jackson and Todd, glucose tolerance tests were performed on 128 cases which were subsequently histologically confirmed as neoplastic. Of these cases, the organ distribution of tumors was as follows: breast 20, genital organs 24, gastrointestinal tract 48, liver and pancreas 12, urinary tract 8, respiratory tract 10, skin 4, and bones 2. These cases were reviewed initially on the basis of glucose tolerance in relation to grading of the tumors along the lines proposed by Broders (1). In 1941, seven years later, they were again reviewed in relationship to life expectancy.

As a matter of record, we first present an analysis of the blood sugar values, obtained by use of the method of Folin and Wu at the zero hour for the entire group. Of the 128 cases, 4 had values in the 60 mgm. range, 21 in the 70 mgm. range, 36 in the 80 mgm. range, 33 in the 90 mgm. range, 23 in the 100 mgm. range, 5 had values between 110 and 120 mgm. and 6 showed values over 120 mgm. To summarize more concisely, 91 per cent of the group had an initial blood sugar concentration of between 60 and 100 mgm. per 100 cc. of blood.

Jackson and Todd have suggested that instead of presenting the glucose tolerance test as a curve, it be presented as a single value, derived by adding the blood sugar values of the zero hour to the values obtained at the 45 and 120 minute periods. This procedure has been followed in the subsequent analyses.

Of the 128 cases, 80 were graded according to Broders (1). Of these, 11 were graded as I and showed a range, according to the Jackson and Todd method of computation, between 453 and 883 mgm. with an average of 674 mgm.; 44 were Grade II and showed a range of from 486 to 1,151 mgm. with an average of 788 mgm.; 18 cases were graded III, these having a range from 504 to 1,125 mgm. with an average of 725; 7 were graded IV with a range of from 527 to 2,167 mgm. and an average of 956 mgm. None of these patients were cachectic; one was a diabetic.

From the data it appears that the fasting blood sugar of the tumor bearer is either within the normal range or possibly slightly below it. While a general statement can be made to the effect that the more anaplastic the growth the less the ability of the host to free the blood stream of excess sugar, this can only be applied to a consideration of group behavior. It cannot be applied to individuals because of the wide fluctuations in the blood sugar values.

The final analysis concerned the relationship of glucose tolerance to life expectancy. Of the 128 patients, 86 have been successfully followed up—21 died within a year, and these showed a range between 508 and 1,255 mgm. with an average of 805 mgm.; 21 patients died in the second post-operative year, the range being 504 to 2,167 mgm. with an average of 794 mgm.; 17 patients died in the third post-operative year, and these had a range of from 510 to 2,189 mgm., with an average of 838 mgm.; 8 died in the fourth post-operative year, and had a range between 528 and 836 mgm., with an average of 698; in the fifth post-operative year 8 died and their averaged tolerance was 814 with a range between 581 and 1,151 mgm.; 11 are still alive in the sixth post-operative year with a range of from 453 to 929 mgm. and an average of 722 mgm. Other analyses were made in an attempt to establish relationships between the type of tumor and sugar tolerance, and between the site of the tumor and sugar tolerance. No relationship could be demonstrated.

* This investigation was aided by a grant from the Amidon Fund.

Conclusions

No relationship between the degree of malignancy and glucose tolerance in individual cases could be established in a study of 128 cases over a period of 6 years. If group behavior is considered, it appears to be true that groups having the more anaplastic tumors do not rid the blood stream as quickly of an excess of glucose as do groups carrying the less anaplastic tumors. This assertion is based upon a consideration of group averages. The glucose tolerance of the tumor bearer furnishes no data of prognostic value as to the expectancy of life of the tumor bearer.

REFERENCES

1. Broders, A. C. Grading of Carcinoma. Minnesota Med., **8**:726-730. 1925.
2. Jackson, D., and D. A. Todd. Sugar Tolerance in Cancer with Reference to Degree of Malignancy. Texas State J. Med., **30**:197-203. 1934.

Abnormal Paramecia Produced by Blastogenic Agents and Their Bearing on the Cancer Problem*†

J. C. Mottram, M.B.

(From Mount Vernon Hospital, Northwood, Middlesex, England)

(Received for publication November 16, 1940)

This research is the outcome of the thought that fresh light might be thrown on the cancer problem if cells which could be handled as single cells were subjected to tumor-producing agents. For this purpose, *Paramecium aurelia* was chosen because it and the closely related species, *P. caudatum,* have probably been more under observation than any other unicellular organisms.

Watch was kept for changes which could be likened to the occurrence of tumors in the higher animals. Such a change has been found in the occasional production of abnormal cells which on reproduction by cell division, after removal of the agent, gave rise to populations of cells having wide morphological variation resembling in many respects the assemblages of cells of which tumors are built.

Abnormalities in the protozoa have been recorded by many observers.[1] Jollos (13, 14), Dawson (7, 8, 9), Jennings (12), and Moore (16) described abnormalities of unknown origin. Abnormalities produced by cutting the cells into parts were observed by Peebles (21) and Calkins (3). Abnormalities beginning at cell division have been reported by Popoff (22), Calkins (2), and Stocking (25). Abnormalities beginning as double cells were described by Dawson (6), DeGaris (10), Simpson (24), Calkins (4), Chatton (5), and Reynolds (23).

The following generalizations are drawn from the reports of these authors:

I. These abnormalities do not breed true. They give rise to mixed populations of cells varying from normal, or apparently normal, through many grades and types of abnormality to monsters.

II. Proneness to abnormality instead of a particular abnormality is passed to descendants.

III. By repeated selection of normal cells from the mixed populations, clones can be produced composed almost entirely of normal, or apparently normal, cells. But when such normal cells are grown in mass culture a very small percentage of abnormals is usually found. By repeated selection of abnormal cells clones composed entirely of abnormal cells result, and the degree of abnormality can thus be increased. In a few cases, abnormals thus produced have bred true for abnormality for many generations.

IV. Selection does not cure proneness to abnormality; it only eliminates it.

V. When grown without selection normal cells usually gain the upper hand and finally, after several subcultures, almost entirely replace the abnormal. Nevertheless, many normal cells have growth rates as high as normal individuals. The greater the degree of abnormality the less the viability. Large monsters often die without reproduction.

VI. It is probable that these abnormalities always begin as doubles, or trebles, or multiple monsters, due to the failure of the cells to separate into daughters at cell division; *i.e.,* failure of cleavage. My own observations greatly favor this view.

VII. There is evidence that a change in the cytoplasm, rather than any nuclear change, is a factor of importance in

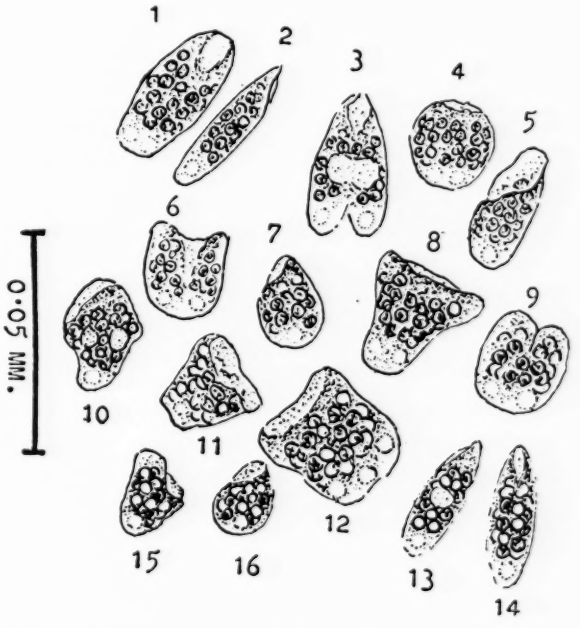


FIG. 1.—The appearance of living *Paramecia* in a strong solution of gum acacia. The dotted circles are contractile vacuoles; the other circles are food vacuoles. The macronucleus in 3, 10, and 13 is shown dotted with a full line margin. Nos. 13 and 14 are normal individuals. No. 2 is a side view of No. 1. No. 9 shows No. 8 contracted. Nos. 8, 11, and 12 swim like *Vorticella;* the others swim in wide irregular spirals.

* The expense of this research was partly defrayed by the British Empire Cancer Campaign, and apparatus bought out of a grant made by the Fitton Trust.

† The manuscript of this paper was transferred to *Cancer Research* by the *American Journal of Cancer.* In order to avoid delay caused by the war the author has permitted the Editorial Committee to revise the manuscript and to publish the paper without submission of the revision to him.—Editor.

[1] Dr. Mottram's detailed review of these observations has been omitted.—Editor.

the failure of cells to separate at cell division and in the production of abnormal cells. It will be seen that my own observations favor this view. Although the original change may be cytoplasmic it is obvious that subsequent unequal and abnormal cell division may lead to nuclear abnormalities. In the production of these abnormal cells, however, the fault in the first place seems to lie in cleavage and not in nuclear division.

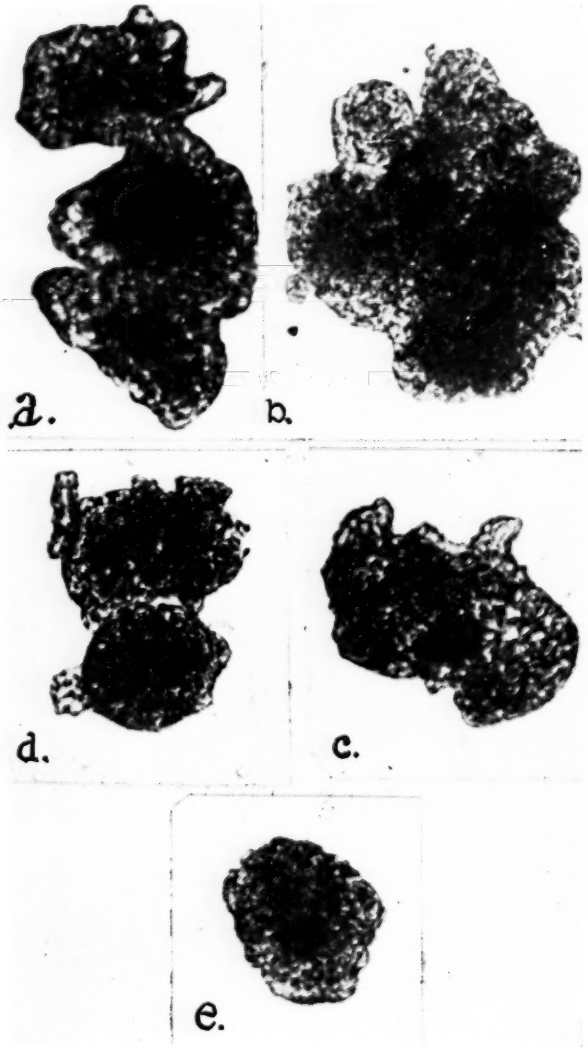


FIG. 2.—(a) From a 1,2,5,6-dibenzanthracene culture. (b) From a cholanthrene culture. (c) From a culture exposed to gamma radiation. (d) From a culture exposed to ultraviolet radiation. (e) From a heated culture. Mag. × 500.

EXPERIMENTAL

THE LIFE HISTORY OF ABNORMAL CELLS PRODUCED BY CARCINOGENIC AGENTS

The origin of abnormal individuals is not dealt with in this section. The observations to be recorded here are concerned with the way in which the abnormal cells behave after being picked out from experimental cultures and transferred to normal media.

In all these experiments, the normal medium consisted of equal parts of distilled water and Peter's solution, containing 15,000 million *Staphylococcus albus* per cc.

Some of the more common types of abnormality of *Paramecium aurelia* are shown in Fig. 1.

Normal individuals vary much in size and shape according to whether they are either filled with, or empty of food. In Fig. 1, Nos. 1 and 2 are different

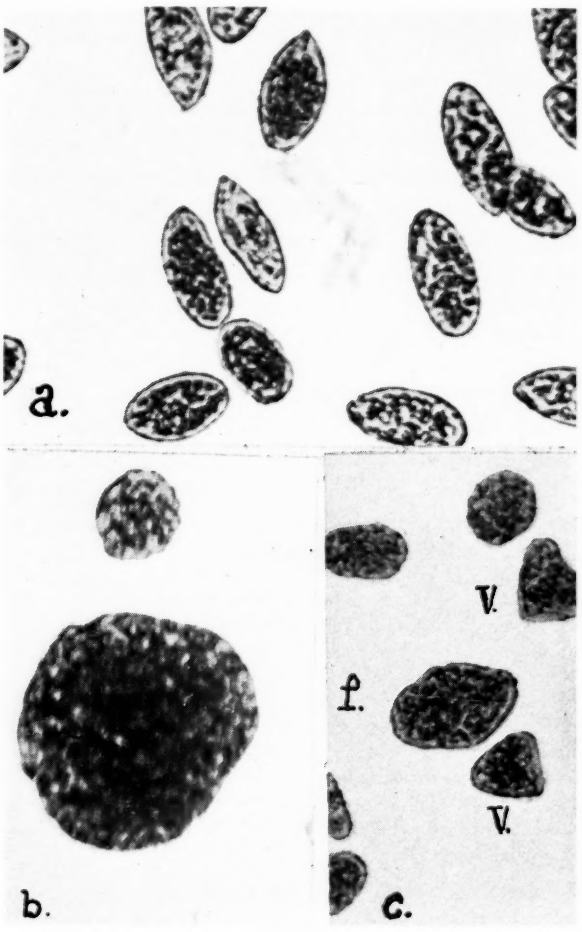


FIG. 3.—(a) Part of a normal culture of *Paramecium*. Mag. × 500. (b) Part of an abnormal culture resulting from an abnormal cell picked from a culture exposed to cold, showing a macro- and a micro-form. (c) Part of a culture resulting from an abnormal cell picked out from a 3,4-benzpyrene culture. Mag. × 300. (f) A "flat." (V) "Vorts."

aspects of a flat form which swims through the water like a revolving dinner plate. Often the posterior end is notched. It is a semi-twin in which the posterior end is more or less double. I have named Nos. 8, 11, and 12 (Fig. 1) "vorts" because they swim like free *Vorticella*. The anterior end is broad and invaginated; they represent anterior semi-twins. Nos. 5, 7, 15, and 16 (Fig. 1) are micro-forms which spin through the water like *Volvox*. They show slight anterior invagination. No. 5 (Fig. 1) is a large form which resembles

a shell. It is invaginated laterally and swims in wide spirals. Between these types all kinds of intermediate forms are found.

Some of the monsters found in experimental cultures and portions of the abnormal populations to which they give rise on reproduction are shown in Figs. 2 and 3.

The following are a selection of the experiments carried out, to show in detail the kind of observations which were made. The first example shows the production of a mixed population from a single abnormal cell and how, by selection, a race of cells all abnormal was produced, which bred true for abnormality but not for any particular type of abnormality.

On 23/11 an abnormal cell was picked out from a 1 in 500,000 benzpyrene culture. On 24/11 this had produced a mixed population of normals and various abnormals. A vort was picked out and then a number of subcultures were made, always picking out a vort, until 29/11 when a tiny micro was picked out. On 2/12 the resulting population was found to consist of nothing but abnormals. Subcultures were frequently made by picking out micros, until 7/12 when two micros were picked out, called Micro A and Micro B. Micro A was kept going by picking out. Micro B was now subcultured, using a platinum loop, 9 times between 7/12 and 31/12. On 31/12 no normals could be found, only abnormals varying much in size and type. On 16/12 from Micro B, the most normal specimen which could be found was picked out; this was grown until 8 granddaughters had resulted and these were separated. Fig. 4 shows that no normals were produced. In this and all subsequent experiments, all culturing was made in normal media. The results showed that continued exposure to the carcinogenic agents was not necessary for the subsequent reproduction of abnormal cells. Micro A, kept going by picking out, was still giving rise to abnormals when discontinued on 31/12.

The second example shows how selection failed to eliminate the normals and how, on propagation without selection, normals gained the upper hand.

A vort was picked out from a 1 in 1,000,000 benzpyrene culture on 1/12. It was subcultured by picking

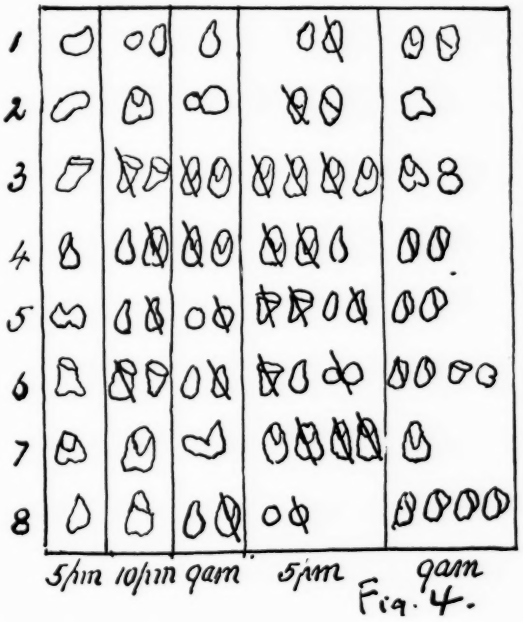


Fig. 4.—Diagram to show how the 8 granddaughters of cell Micro B reproduced during 40 hours. The organisms crossed out were removed from the cultures. In the case of No. 5 three abnormals were removed at 5 p.m. on the 2nd day leaving one apparently normal cell. This was shown to be abnormal as its descendants at 9 a.m. on the following day were 2 "shells." Likewise in No. 4 at 10 p.m. and at 5 p.m. an apparently normal was left but only abnormals resulted on reproduction. Thus attempts to produce normals by selection failed.

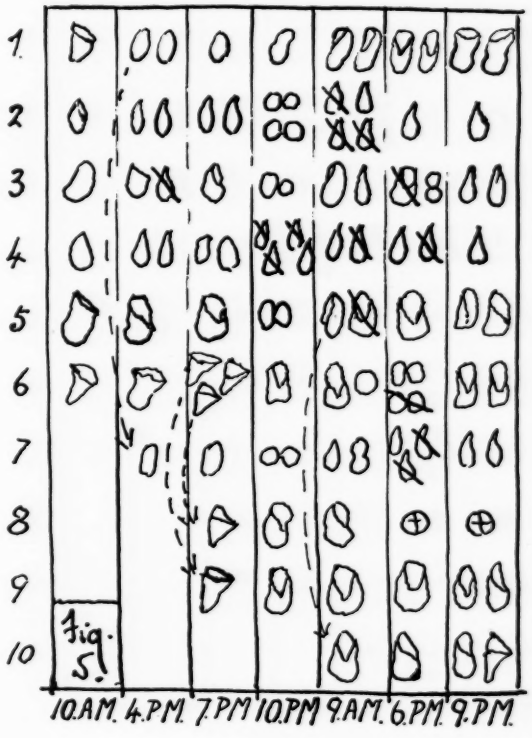


Fig. 5.—Diagram of the growth of 6 organisms from a benzpyrene culture. Arrows indicate the cells chosen to make the clones up to 10. Cells crossed out were removed. The last column characterizes the populations on the morning of the 3rd day. Nos. 2, 4, and 7 were normal from the start and continued so. In the mixed populations normals appeared later.

Populations at 10 a.m. next morning: Line 1, Abnormals; Line 2, Normals; Line 3, Mixed; Line 4, Normals; Line 5, Mixed; Line 6, Abnormals; Line 7, Normals; Line 8, Cysts; Line 9, Mixed; Line 10, Mixed.

out vorts on 2/12, 5/12, 7/12, 10/12, 11/12, and 14/12. On 14/12 two vorts were picked out, called Vort A and Vort B. These gave mixed populations of abnormals and a few apparently normals. Vort A was subsequently subcultured using a platinum loop on 20/12, 22/12, 24/12, 26/12, and 29/12. On 20/12 the organisms were all abnormal. On 21/12 a few apparently normals were seen. On 26/12 normals were numerous and on 31/12 there were far more normals than abnormals. On 22/12 an apparently normal was picked out and kept until it had divided into eight. The eight were separated and grown into large populations, which on examination were found to consist entirely of normal cells. Vort B was cultured by picking out vorts until 31/12, when a population consisting of mixed abnormals was produced, with a

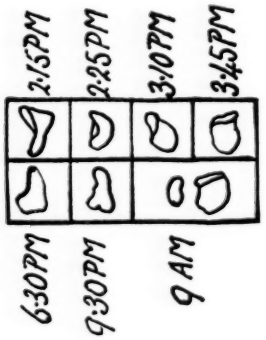


FIG. 6.—This shows the growth of a cell from a 1,2,5,6-dibenzanthracene culture in which cleavage failed, a double resulting. This reorganized into a cell which divided during the night, giving rise to one cell which produced a population of normals, and one which died without dividing.

very small percentage of normals. There was failure to produce a race of pure abnormals.

The next example shows the immediate progeny of a double taken from a benzpyrene culture, and that races of abnormals and normals were quickly separated by selection. The double was cultured until 6 were present, these were then separated and their subsequent history is shown in Fig. 5.

The fourth example is of a double found in a 1,2,5,6-dibenzanthracene culture, in which the resulting abnormality died out after a single division, as shown in Fig. 6.

The fifth example gives the history of a large "flat." It shows that abnormality, rather than one type of abnormality, is transmitted and that the mixed population produced contains normals as well as abnormals.

On 1/12 a large flat, picked out from a 1 in 1,000,000 benzpyrene culture, was subcultured by always picking out a flat until 14/12. By that time populations of vorts, flats, shells, and other abnormals were being produced, with a few normals. On 13/12, a flat was picked out and cultured as a side line. It grew into 1 flat, 2 shells, 4 vorts, and 8 apparently normal cells. The 8 normals were made into single cell cultures. Of these, 3 gave mixed populations of normals and abnormals, 4 produced populations of normals, and 1 died. Returning to the original line, on 14/12 subculturing by platinum loop was begun and carried on until 20/12, by which time many normals were present in the culture. Eight apparently normal cells were picked out and grown as separate clones. In all 8 cases populations of normals resulted, containing no abnormals. Subculturing with a platinum loop was continued. The large flats and other abnormals gradually became less, on 26/12. A few large flats were still present. By 30/12 no abnormals were found. Normals had completely displaced them.

In the next example is given the short history of two doubles, the result of cells which failed to separate at cell division and which were found in a 1 in 1,000,000 benzpyrene culture. As shown in Fig. 7 they first reorganized into single cells, and then gave rise to abnormal cells.

The only 2 abnormal cells observed among control cultures, during about 2 years, were taken from a control culture to cholanthrene exposures. One behaved as shown in Fig. 8, going quickly back to 4 normals.

An abnormal derived from gamma ray exposures and picked out on 10/1, had the history shown in Fig. 9. It is evident that the abnormals so produced behave like those resulting from exposure to the blastogenic hydrocarbons.

Likewise abnormals produced by ultraviolet radiation are no different from abnormals produced in other ways. Two doubles were picked out on 10/1 and grew as shown in Fig. 10.

An example of abnormals produced by cold clearly shows their origin from doubles. Four dividing cells were found in a culture which had been subjected to

DESCRIPTION OF FIGURES 7 TO 9

FIG. 7.—Drawings of 2 cells from a benzpyrene culture which failed to cleave but which after a long time divided and gave rise to abnormal cells.

FIG. 8.—Diagram showing how a failure to cleave was quickly rectified. Cells from a control culture.

FIG. 9.—The uppermost set of drawings shows the growth of the abnormals produced in a culture exposed to gamma ray. After 48 hours 8 cells were present. Of these 8 cells, 6 were separated (1 to 6) and grown as single cell cultures. Nos. 1, 2, and 3 were fixed on 14/1 and the individuals present are shown in the middle section of the chart. Nos. 4, 5, and 6 were fixed on 15/1 and are similarly dealt with in the lowest section of the chart. Mixed populations resulted containing very few normals.

Fig. 7.

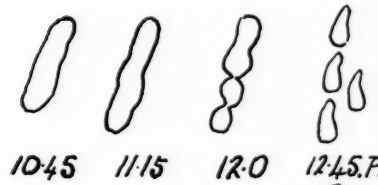
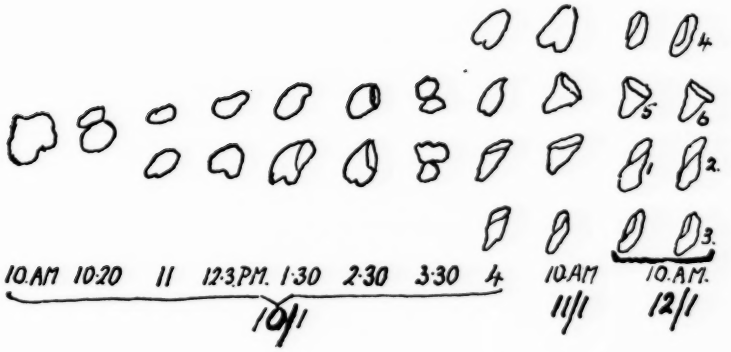


Fig. 8



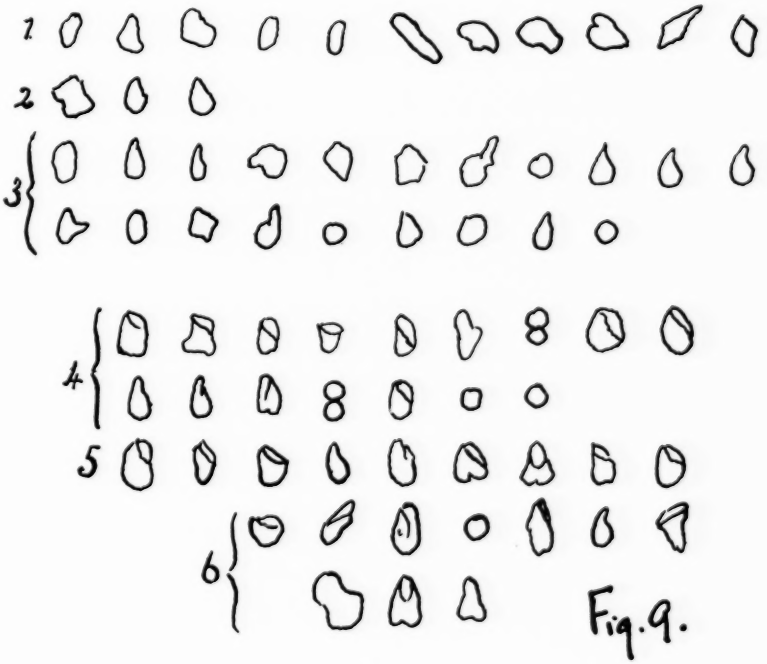


Fig. 9.

FIGS. 7-9

cold. These showed greatly delayed division. They were separated and their subsequent history is shown in Fig. 11.

These cells, as shown in Fig. 11, reorganized into single cells which did not divide for 24 hours. They then divided, giving 11 cells which were cultured as single cell cultures. These gave rise to populations containing abnormal as well as apparently normal cells, as follows:

No. 1 contained 3 abnormal and 5 normal; No. 2 contained 1 abnormal and 3 normal; No. 3 contained 12 normal; No. 4 contained 5 abnormal and 6 normal; No. 5 contained 4 abnormal and 4 normal; No. 6 contained 1 abnormal and 7 normal; No. 7 contained 3 abnormal; No. 8 contained 3 abnormal and 2 normal; No. 9 contained 5 abnormal; No. 10 contained 1 abnormal and 14 normal; No. 11 contained 4 abnormal.

Finally the behavior of an abnormal cell produced by heat shock is shown in Fig. 12. The cell when first seen was in very unequal division.

The final population consisted entirely of very abnormal forms, one consisting of a huge knobbly monster which spun slowly around at the bottom of the well slide.

The conclusions to be drawn from these observations are the same as those which were summarized in the first portion of this paper as a result of considering the literature of abnormalities in the protozoa. The only addition required is the statement that the continued exposure of the blastogenic agents which produced the abnormal cells is not at all necessary for their reproduction, and further, that abnormals so produced will continue to produce abnormals, at least for many generations, if not indefinitely.

The Occurrence of Abnormal Cells in Cultures of Paramecium Exposed to Blastogenic Agents

Five agents have been applied: 1. the blastogenic hydrocarbons; 2. gamma radiations from radium; 3. ultraviolet radiation from a mercury vapor lamp; 4. cold; and 5. heat.

Ultraviolet radiations.—An unscreened quartz mercury vapor lamp was used. Cultures are exposed daily at a distance of 30 cm. In a first series, 6 cultures were exposed for ⅛, ¼, ½, 1, 2, and 4 seconds daily with 2 controls. Accuracy of exposure was obtained by using a revolving disc of cardboard out of which a section had been cut. Those exposed for 4 seconds ceased to divide on or about the 5th day, after which they gradually died, all being dead on the 48th day. The ⅛-second and ¼-second specimens were discarded on the 23rd day, having shown no departures from the normal. The ½-second specimen was continued to the 41st day, when an abnormal cell was found, and the 1-second specimen first presented an abnormal cell on the 20th day. In the 2-second specimen, abnormals were found on the 12th day and the culture was discarded.

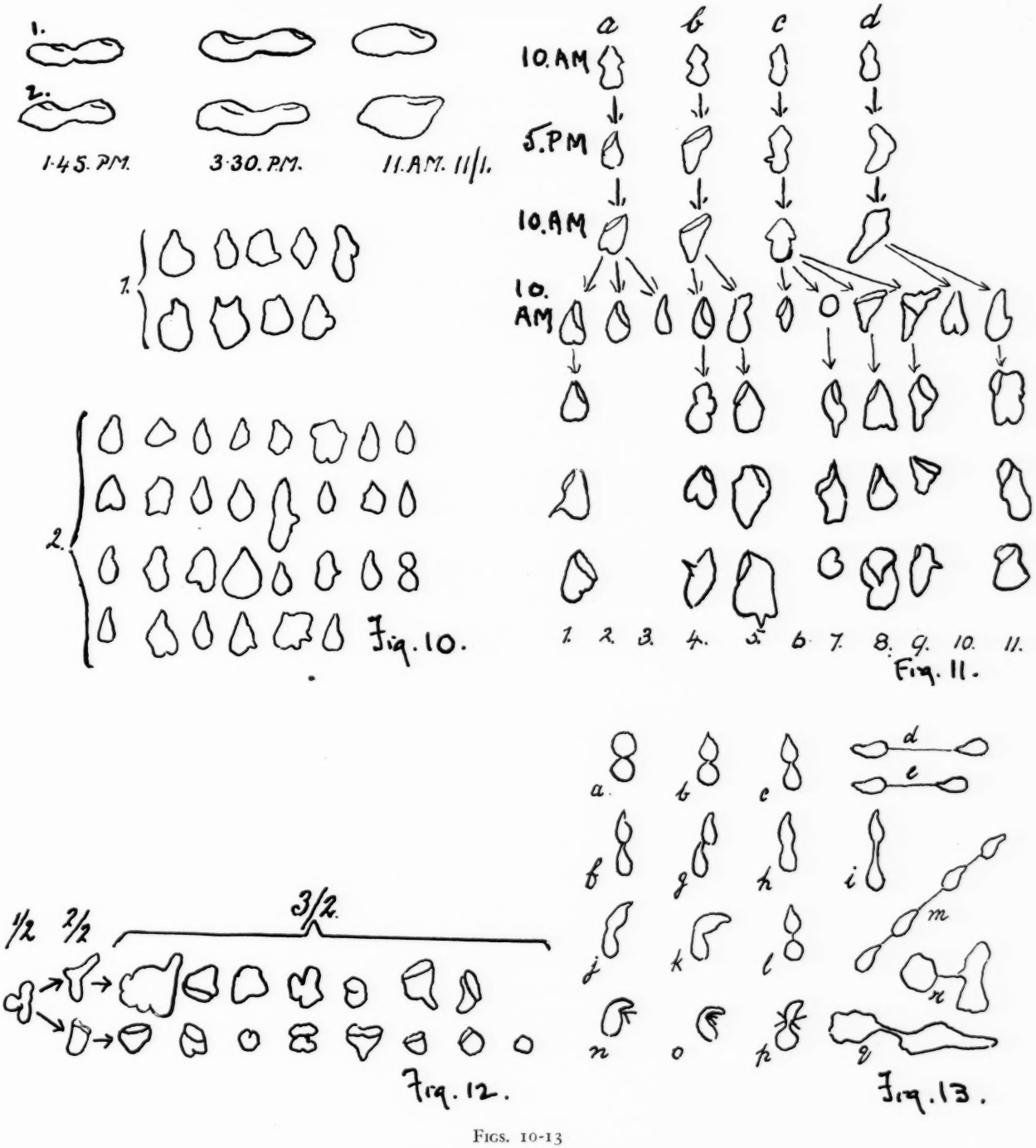
Additional 1-second and 2-second cultures were made, and altogether 7 abnormals were produced as follows: ½ second daily, abnormals on the 41st day; 1 second daily, abnormals on the 14th, 20th, and 32nd days; 2 seconds daily, abnormals on the 11th, 12th, and 20th days.

The abnormals were picked out in all cases and grown in normal medium for a few generations, to make sure that they could reproduce abnormality. In many cases throughout these experiments the abnormal cells when first seen were, without doubt, cells which had attempted to divide and had failed and which then reorganized themselves into a single abnormal cell. Four out of these 7 abnormal cells were seen to begin as doubles. The rest were not seen early enough to determine how they first began. In 6 control cultures observed for 50 days, no abnormals were seen. Cultures made of media exposed to ultraviolet radiation did not produce abnormals.

Gamma radiation.—Cultures were exposed between 2 flat radium applicators screened with lead and aluminum so as to give pure gamma radiation. The intensity was 18.5 r a minute. Daily exposures were made, omitting Sunday, for 1.1, 4.4, and 17.6 minutes, equivalent to 20, 80, and 320 r per day. Two cultures served as controls. The first abnormal cell was found in the 320 r culture on the 4th day. Subsequently an exposure of 160 r was also made. When once an abnormal cell had occurred in a culture, it was discarded and a new culture begun. Altogether 6 abnormal cells were thus produced as shown in the following summary: 20 r daily, abnormal on the 40th day; 80 r daily, abnormal on the 32nd and 46th days; 160 r daily, abnormal on the 62nd day; 320 r daily, abnormal on the 4th and 38th days. Two control cultures not radiated, no abnormals in 50 days.

In all cases where the beginnings in production of abnormal cells were obtained, it was seen to arise in the failure of daughter cells to separate at cleavage.

Exposure to cold.—The experiments of Berenblum (1) and Mansens (15) clearly show that tumors can be produced by exposing the skin of mice to carbon dioxide snow. On this account, cold has been employed in these investigations. It was found that the exposure of cultures to a temperature of 3° C. resulted in the production of abnormals. One slide was placed at 3° C. overnight and then removed to room temperature; the other kept at room temperature served as a control. In the morning the cold culture presented many cells in which division had begun but had been held up near its termination. No early stages of cell division were found. The cold culture is now kept at



FIGS. 10-13

FIG. 10.—The uppermost section of the chart shows the reorganization of the undivided cells into single (double) cells from abnormals produced in a culture exposed to ultraviolet light. No. 1 was permitted to grow until 13/1, when the culture was fixed; the individuals found are shown in the middle section of the chart. No. 2 was fixed on 14/1; the resulting individuals are shown in the lowest section of the chart.

FIG. 11.—Diagram of cells derived from a culture exposed to cold, showing the growth of 4 doubles (a to d) during 48 hours when 11 cells were present. These were made into single cell cultures which were fixed 24 hours later. Some of the abnormals produced are shown in the lower portion of the chart.

FIG. 12.—Diagram showing the growth of an abnormal from a culture exposed to heat shock. During 48 hours 15 individuals were produced. All were abnormal; many were monsters.

FIG. 13.—Various abnormal cleavages of *Paramecium* are shown in this diagram. Types q and r are from Stocking (25); n, o, and p deal with flexibility.

room temperature, when it will be seen that most of the cells delayed in division quickly proceed to divide into 2 normal cells. A very few, however, reorganize themselves into single cells about twice the size of the normal *Paramecium*. These are doubles. If these cells are picked out and watched, it will be found that they will not divide again for a long time, often as long as 48 hours. The normal division rate at 15° C. in cultures in fresh Staphylococcus-Peter's medium, is about 5 to 6 hours. When after this delay the doubles divide, 2 cells unequal in size result. Further divisions lead to the production of the mixed populations already described. This is a certain and easy way of producing abnormal cells in *Paramecium,* which behave exactly like those produced less easily in other ways.

If old cultures are used containing no dividing cells, then no abnormal cells are produced by exposure to cold, showing that the abnormal cells are related to cleavage as well as to exposure to cold.

Exposure to heat.—In applying heat, the exposure must be such that the injury does not inhibit cell division because cell division is necessary for the production of abnormal cells. To apply such a precise exposure to many *Paramecia* is perhaps difficult. But by plunging a red hot platinum wire into a rapidly growing culture, some cells will be killed and others receive varying sublethal doses, sometimes so small as not altogether to inhibit mitosis. The cultures were so treated with 1, 3, and 6 plunges of the hot wire once a day. Many cells were thus killed and were eaten by the survivors; some were only injured since they became round but continued slowly to revolve lying on the bottom; many appeared to have entirely escaped injury.

Abnormal cells were found in these cultures. They were picked out and grew into the usual mixed populations. The day of occurrence of abnormals is given as follows:

1 plunge of the wire daily, abnormal on the 15th day;

3 plunges of the wire daily, abnormal on the 10th and 29th day;

6 plunges of the wire daily, abnormal on the 3rd, 6th, 8th, 15th, and 29th day.

In 2 control cultures there were no abnormals in 30 days.

Exposure to blastogenic hydrocarbons.—These agents have been left to the last because for them special precautions must be taken. It has been shown by Mottram and Doniach (19, 20), and by Doniach (11) that these hydrocarbons are photodynamic, therefore cultures of *Paramecium* containing them must be kept in the dark and examined in light screened by a 2a Wratten filter. Colloidal emulsions in glass-distilled water were employed. Since increased cell division (Mottram 17, 18) had been found to occur using 3,4-benzpyrene in a dilution of 1 in 1,000,000, dilutions of this order were used. The hydrocarbons given in Table I were used in dilutions of 1 in 200,000, 1 in 500,000, and 1 in 1,000,000. In the controls, water replaced the emulsions of the hydrocarbons. When abnormal cells were observed, the cultures were discarded and fresh specimens made in their stead. Wherever an abnormal cell was found it was picked out and not counted as an abnormal unless it gave rise to the usual mixed abnormal population. The results are shown in Table I.

Table I: Effects of Blastogenic Hydrocarbons Upon Paramecium During 80 Days in Culture *

Hydrocarbon	Dilutions 1-1,000,000 Abnormals	1-500,000 Abnormals	1-200,000 Abnormals
9,10-Dimethyl-1,2,-benzanthracene	None	61	7.67
Cholanthrene	3.8.61	None	None
3,4-Benzpyrene	13.60	None	None
1,2,5,6-Dibenzanthracene	66	18.20.39	7
1,2-Dibenzanthracene	None	None	None
1,9-Dimethylphenanthrene.	None	12.75	7.62.75
Anthracene	None	None	None
Cholesterol	None	None	None

* Eight control cultures did not produce any abnormals in 80 days.

Other Changes in Paramecia Produced by Blastogenic Agents

Delayed separation of the daughter cell at the end of division.—It has been seen, both in the literature and in my experiments, that abnormal cells usually if not invariably begin in the failure of cells to separate into two at cell division. For this reason, delay or difficulty in cleavage has been looked for in the experimental cultures.

When fully fed normal individuals divide, the 2 cells at the moment of separation are round and equal in size as shown in Fig. 13a. When less fully fed, the anterior cell or the one which is always in front when swimming, is pointed anteriorly (Fig. 13b). When they are more or less empty of food vacuoles at the time of division, both are pointed as in Fig. 13c.

In the experimental cultures, the pointed or more differentiated form is much more prevalent. Even with fully fed individuals, single pointed and often double pointed doubles are to be seen. In specimens not fully fed and more or less empty of food, differentiation is carried further before separation occurs, as shown in Fig. 13f, when the cells assume almost adult characters before separation. It is to be noted that in extreme cases, the posterior individual is attached a little way up the back of the anterior cell and not at its end as in normality (Fig. 13g).

These observations suggested that there is delay in separation which allows a near approach to adult characters before separation.

Cells were therefore picked out when they first showed signs of cell division and note made of the time taken till the daughters separated. For normal cells the average time in 22 cases was 22 minutes; minimum, 13 minutes; maximum 29 minutes. For experimental cells, times above 45 minutes were common; above 60 minutes, frequent, and several hours, occasionally. Cells which reorganize to double cells do not separate. These are more solidly joined, as shown in Fig. 13, h and i; often the anterior cell is joined at an angle to the posterior (Fig. 13, j and k).

The degree of delayed separation gradually increases in the experimental cultures until finally double forms are found. In the experiments with cold, delayed division is frequent and double cells can often be picked out, in which division is so delayed that it is uncertain whether it occurs. Often the dividing cell can be observed to reorganize into a single double which, as already described, is the beginning of abnormal cells.

In the experiments with hydrocarbons, the findings indicate that preceding for a long time the production of abnormal cells, the experimental cultures show that there is some delay and difficulty in the separation of the daughter cells at the end of division.

The phenomenon of the thread formation.—When normal daughters break apart, occasionally for a moment after separation an extremely fine thread can be seen joining the cells, which instantly snaps (Fig. 13l). In the experimental cultures, this thread often persists for from a few seconds up to many minutes, the anterior cell towing the posterior by an extremely fine cytoplasmic (ectoplasmic) connection. As time passes, the thread grows longer and may be as much as three times the length of the cell (Fig. 13d). A common length is twice the length of a cell (Fig. 13e). The thread is elastic, stretching when the anterior cell makes a spurt forwards and then contracting to bring the posterior cell forward as almost to ram the anterior. When the 2 cells revolve in opposite directions, as they often do, the thread does not appear to twist up. Very rarely, the thread is much thicker, and then the cells seemed to be joined by a rigid stick of cytoplasm (Fig. 13i). On one occasion I found a double cell in an experimental culture which gave rise to 3 threads (Fig. 13m). These threads have frequently been observed in all experimental cultures where abnormals have occurred, apart from the experiments with cold where sticks instead of threads are as often seen.

Other observers, Stocking (25) and Dawson (6), have noted these threads in association with the occurrence of abnormals (Fig. 13, q and r). Their presence shows that the ectoplasm of the cell is abnormal. Further evidence that perhaps the ectoplasm has been altered is seen in cultures treated with cold or with heat. In the chilled cultures many cells are often seen containing enormous contractile vacuoles, as if they could not break through the ectoplasm. When these cultures are warmed, the vacuoles break after a time and subsequently they function normally. Similar cells are found in heated cultures. These also recover after a few hours.

The flexibility of the cytoplasm.—The normal *Paramecium* is rigid except for its anterior end which is often bent to an angle of 45° in passing round an obstruction, as in Fig. 13n. The anterior end can be pushed through a narrow chink but the rigid body will not follow and the organism then backs out.

In the abnormal cultures produced by agents other than heat and cold, greatly increased flexibility is seen, beginning long before abnormal cells appear, and it is very marked at the time of their appearance. This flexibility is seen sometimes when the organism is swimming, when it will bend upon itself to form a U-shape. It is, however, best seen when the animals are feeding on the bottom and meet obstructions there. In negotiating a corner they will bend around it, as in Fig. 13, o. Also they will pass between two particles very close together, squeezing gradually through, as in Fig. 13p. It may take a minute or more for the cell to pass and during this time the cell contents can be seen streaming past the obstruction like sand in an hour glass. When these flexible *Paramecia* are thus occupied, they can easily be mistaken for small amebae. The state of flexibility is therefore further evidence that the cytoplasm has been altered. Dawson (6) also observed increased flexibility in association with abnormal *Paramecia* and considered that this was evidence of a peculiar state of the protoplasm. To this state he attributed invaginated forms and these have been very common in the abnormals of my experiments.

My impression is that increased flexibility of the cytoplasm allows invagination to occur. There is no doubt that these changes, flexibility, invagination, failure of the contractile vacuole to break, the presence of a thread, and delay or failure in cell division, all have a common cause located in the cytoplasm which contains the clue to the production of abnormal cells and, by analogy, is probably of importance in the etiology of tumors.

The Bearing of These Results on the Cancer Problem

The prime object of this research was to see whether blastogenic agents would effect any changes in a unicellular organism equivalent to tumors in vertebrates.

The answer is given in the following tabulated summary:

Tumors in Vertebrates	Abnormal Cells in Paramecium
1. Induced by blastogenic agents.	1. Produced by 5 of these agents comprising chemicals, radiations, heat, and cold.
2. Occasionally follow simple wounds.	2. Produced by cutting experiments; *i.e.*, the removal of a part of the cytoplasm.
3. Occur in actively growing tissues rarely, if ever, among non-dividing cells; often hyperplasia precedes tumor formation.	3. Occur only in cultures containing dividing cells because they begin in a failure of cleavage; in the case of benzpyrene increased growth rate precedes their occurrence.
4. Occur in a punctate manner; only a very few of the treated cells become tumor cells.	4. Are to be found in only very small numbers in the treated cultures.
5. Once produced will continue to grow in the absence of the blastogenic agent.	5. Will produce populations of abnormal cells in the absence of the agents.
6. Can be propagated indefinitely by grafting without loss of the ability to form tumors.	6. Can be cultivated indefinitely without loss of the capacity to produce populations of abnormal cells.
7. Consist of cells showing wide morphological variation.	7. Produce populations of cells varying widely from apparently normal to monstrous forms.

Summary and Conclusions

Paramecia were subjected to five blastogenic agents; namely, polycyclic hydrocarbons, gamma radiation, ultraviolet radiations, heat, and cold. Occasionally abnormal cells resulted which, on reproduction in the absence of these agents, produced populations of cells varying from apparently normal to very abnormal monstrous forms.

Proneness to abnormality was transmitted rather than any special type of abnormality, and this transmission could be carried on indefinitely. These occurrences resembled in many respects the production of tumors in multicellular animals.

The first change in the *Paramecia* preceding the occurrence of abnormal cells was a change in the cytoplasm. This interfered with cleavage, leading first to delay, and later occasionally to a failure to cleave.

After failure to cleave, the cell reorganized itself into a single cell having a double constitution. The subsequent irregular cell division of these doubles gave rise to the polymorphic abnormal populations resembling the cells of which tumors are composed.

It is suggested that the blastogenic hydrocarbons effect changes in the cytoplasm like those produced by radiations and change in temperature. It is possible that biological changes similar to the abnormalities observed in *Paramecia* are involved in the etiology of tumors.

References

1. Berenblum, I. Tumour-Formation Following Freezing with Carbon Dioxide Snow. Brit. J. Exper. Path., **10**: 179-184. 1929.
2. Calkins, G. N. The Protozoan Life Cycle. Biol. Bull., Wood's Hole, Mass., **11**:229-244. 1906.
3. Calkins, G. N. Effects Produced by Cutting Paramecium Cells. Biol. Bull., Wood's Hole, Mass., **21**:36-72. 1911.
4. Calkins, G. N. Uroleptus Mobilis. V. The History of a Double Organism. J. Exper. Zoöl., **41**:191-213. 1924.
5. Chatton, E. Réversion de la scission chez les ciliés. Réolisation d'individus distomes et polyénergides de *Glaucoma scintillans* se multiplient indéfiniment par scissiparité. Comp. rend. Acad. d. sc., **173**:393-395. 1921.
6. Dawson, J. A. An Experimental Study of an Amicronucleate Oxytricha. II. The Formation of Double Animals or "Twins." J. Exper. Zoöl., **30**:129-157. 1920.
7. Dawson, J. A. Inheritance of an Abnormality of Form in Paramecium Aurelia. Proc. Soc. Exper. Biol. & Med., **22**:104-106. 1924.
8. Dawson, J. A. A Mutation in Paramecium Aurelia. J. Exper. Zoöl., **44**:133-157. 1926.
9. Dawson, J. A. The Mutant Paramecium Aurelia. Science, **68**:258. 1928.
10. De Garis, C. F. A Genetic Study of Paramecium Caudatum in Pure Lines Through an Interval of Experimentally Induced Monster Formation. J. Exper. Zoöl., **49**:133-145. 1927.
11. Doniach, I. A Comparison of the Photodynamic Activity of Some Carcinogenic with Non-Carcinogenic Compounds. Brit. J. Exper. Path., **20**:227-235. 1939.
12. Jennings, H. S. The Effect of Conjugation in Paramecium. J. Exper. Zoöl., **14**:279-391. 1913.
13. Jollos, V. Untersuchungen über Variabilität und Vererbung bei Arcellen. Arch. f. Protistenk., **49**:307-374. 1924.
14. Jollos, V. Untersuchungen über Variabilität und Vererbung bei Arcellen. Biol. Zentralbl., **44**:194-207. 1924.
15. Mansens, B. J. Hautkrebs bei Mäusen durch Einwirkung von Kohlensäureschnee. Acta brev. Neerland., **1**:21-22. 1931.
16. Moore, I. Inheritance of Atypical Form in Paramecium aurelia. Proc. Soc. Exper. Biol. & Med., **24**:770-771. 1926.
17. Mottram, J. C. An Increase in the Rate of Growth of Paramecium Subjected to the Blastogenic Hydrocarbon 3:4-Benzpyrene. Nature, London, **144**:154. 1939.
18. Mottram, J. C. 3:4-Benzpyrene, Paramecium and the Production of Tumours. Nature, London, **145**:184-185. 1940.
19. Mottram, J. C., and I. Doniach. Photodynamic Action of Carcinogenic Agents. Nature, London, **140**:933-934. 1937.

20. MOTTRAM, J. C., and I. DONIACH. The Photodynamic Action of Carcinogenic Agents. Lancet, **1**:1156-1159. 1938.
21. PEEBLES, F. Regeneration and Regulation in Paramecium Caudatum. Biol. Bull., Wood's Hole, Mass., **23**:154-170. 1912.
22. POPOFF, M. Experimentelle Zellstudien. II. Über die Zellgrösse, ihre Fixierung und Vererbung. Arch. f. Zellforsch., Leipz., **3**:124-180. 1909.
23. REYNOLDS, B. D. Inheritance of Double Characteristics in *Arcella Polypora* Penard. Genetics, **8**:477-493. 1923.
24. SIMPSON, J. Y. Observations on Binary Fission in the Life-History of Ciliata. Proc. Roy. Soc. Edinburgh, **23**:401-421. 1901.
25. STOCKING, R. J. Variation and Inheritance in Abnormalities Occurring After Conjugation in Paramecium Caudatum. J. Exper. Zoöl., **19**:387-449. 1915.

The Fate of Spontaneous Mammary Carcinomas in Mice After Simple Biopsy*

R. Lewisohn, M.D., C. Leuchtenberger, R. Leuchtenberger, M.D., and D. Laszlo, M.D.

(From the Laboratories of the Mt. Sinai Hospital, New York, N. Y.)

(Received for publication March 15, 1941)

In a previous paper (1) we reported the disappearance of 38 spontaneous mammary carcinomas in mice which had been treated intravenously with spleen or yeast extracts. These 38 mice represented 30 per cent of the treated animals. No direct treatment had been applied. We understand that direct treatment means injection into the tumor or treatment with x-rays or radium. Indirect treatment consists of intravenous, intraperitoneal, or subcutaneous injections, or oral administration.

When we started our work with spontaneous tumors (strain A, Roscoe B. Jackson Memorial Laboratory), we believed that biopsies might not be necessary. However, after we had worked with this strain for awhile and after consultation with Dr. C. C. Little, we came to the conclusion that biopsies were essential. Therefore, we excluded from our statistics all previous cases which had been treated and healed without preceding biopsies. Since March, 1939, we have performed a biopsy in every animal.

The technic of the biopsy is very simple. The animal is anesthetized with ether and a minute incision is made through the skin. A small particle of the tumor is removed either with a curved scissors or with a scalpel. The skin incision is so small that it is unnecessary to close the opening with sutures. The opening closes automatically. A drop of tincture of iodine is applied to the place of the incision. Even that may be unnecessary. The incision heals in a very short time.

The importance of these biopsies is evident from the fact that among 712 biopsies performed in this laboratory on mice of strain A, 99 (13 per cent) proved to be nonmalignant (cysts, abscesses) or doubtful cases. Among the doubtful cases we included biopsies in which the small amount of tissue made a definite diagnosis of malignancy impossible. We have always removed very minute pieces of tumor tissue for diagnosis, in order to meet possible criticism that the biopsy might cause disappearance of the tumor.

An extensive experience extending over a number of years has confirmed our conviction that it is impossible either by inspection or palpation to make an absolutely correct diagnosis as to malignancy or nonmalignancy of these tumors. Not even a hemorrhagic appearance of these tumors which is considered as a criterion for malignancy by many experienced cancer research workers is pathognomonic. We have ample proof from our biopsy studies that many hemorrhagic breast tumors in mice (strain A or Rockland Farm) are benign.

It may be surprising that we found such a high percentage of nonmalignancy among the strain A animals. Undoubtedly the percentage of definitely nonmalignant tumors among these animals is considerable. In addition we excluded all those cases in which the slightest doubt existed as to the malignant nature of the tumor. Only unequivocal malignancies are included in the group of 712 treated animals. While we may have been too strict in our histological criteria, we are assured that benign and borderline cases were excluded.

Since we reported our results with spontaneous malignant tumors the objection has been raised by some who had not seen our biopsy technic that the disappearance of these tumors might be due not to the treatment but to the biopsy. It has been claimed that a biopsy interferes with the blood supply of the tumor, and thus causes a spontaneous regression. It seemed *a priori* most improbable that the excision of a minute piece of tumor tissue should interfere in any way with the normal growth of these breast carcinomas because these biopsies are taken from the surface of the tumors.

A temporary marked reduction in the size of the tumor is often noted immediately after the biopsy. Many of these malignant tumors are hemorrhagic and contain a considerable amount of fluid. Naturally a tumor of this nature is apt to shrink in size after the evacuation of the fluid during a biopsy. Thus a previously large tumor may for a considerable period of time after the biopsy show a marked reduction in size. However, as will be evident from our statistical data given below, these reductions in the size of the tumors after a biopsy are purely temporary. Ultimately

* This investigation was aided by grants from The International Cancer Research Foundation and the New York Foundation.

the animals die with very large tumors, sometimes two or three times the size of the animal.

It has long been known that these malignant tumors do not grow continuously. They often show temporary reductions in size, sometimes extending over months. These temporary regressions may be noted immediately after the biopsy. However, sometimes they appear a number of months after the biopsy. The early regression is demonstrated in Fig. 1. The original malignant tumor, as pictured in Fig. 1–1 (December, 1939), was very small in April, 1940 (Fig. 1–2). Even in June, 1940 (Fig. 1–3), the original tumor had not increased in size, though a second tumor of considerable size was noted (Fig. 1–4). However, in September, 1940, both tumors had grown to enormous size and the animal succumbed.

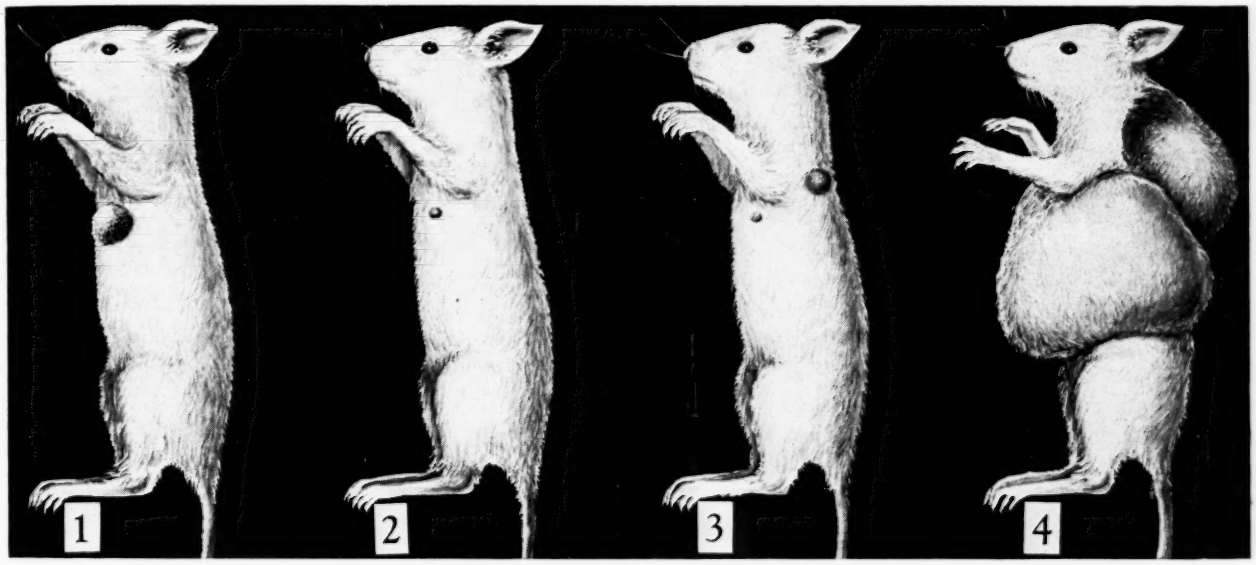


FIG. 1—No. 407. Marked temporary regression of original tumor over a period of 6 months—appearance of second tumor—enormous growth of both tumors.

On account of these temporary changes in size during the life cycle of these malignant tumors we have strictly omitted from our statistics and consideration those cases in which treatment caused merely regressions but not complete disappearance of the tumor. The observation pictured in Fig. 1 proves the importance of such a critical approach. If this animal had received treatment and had died in June, one might have argued that on account of the treatment the tumor had practically disappeared. Yet here was a control tumor which showed the same reduction in size over a period of many months.

Of the 38 healed mice referred to in the report presented before the American Association for Cancer Research in Pittsburgh, in March, 1940, 34 came from the Roscoe B. Jackson Memorial Laboratory (strain A) and 4 only were tumor-bearing mice which we had received from the Rockland Farms. Our present report on controls deals only with the strain A mice.

We shall not discuss here the life expectancy of these strain A mice with mammary carcinomas, the behavior of the cancers or other points of clinical or pathological interest. These tumors have been studied in great detail at the Roscoe B. Jackson Memorial Laboratory for many years. Thus a presentation of many facts which we observed during our studies would be a repetition of previous observations. On the other hand, the effect of biopsies on these or other malignant tumors has not received any detailed studies. For this reason we shall confine our remarks to questions which have a bearing on the possible effect of biopsies of tumors in strain A mice.

Eighty-nine mice of strain A, in which a biopsy performed according to the technic described revealed unequivocal malignancy, were taken as controls. No treatment of any sort was given. Among these control animals 8 died within 2 weeks after the biopsy. The tumors of these animals had increased in size (as compared to the size at the time of the biopsy) when they died of intercurrent infections. We excluded these animals from our statistics, as the observation period was too short to draw any conclusions as to the effect of mere biopsies on spontaneous tumors.

Among the 81 mice which form the basis of this report, 37 showed continuous growth of the tumor until they died with large tumors. Sixteen animals showed,

in addition to the steady growth of the original tumor, appearances of second, third, or fourth tumors. These additional tumors presented the same microscopic pictures as the original tumors. An interesting group of 12 animals showed an initial reduction in size, which often lasted for a few weeks or even months. Ultimately these animals died with large tumors.

While these three groups comprise the majority of the cases (65 among 81 animals) we observed a number of varieties which did not fit into any of these groups. For instance, one tumor (animal No. 436) was stationary for 3 months following the biopsy and suddenly started to grow rapidly. Before the animal died (4 months after the biopsy) a second tumor had appeared.

Another tumor (animal No. 443) was stationary for 6 weeks. Two months after the biopsy the tumor was very small (about one tenth of its original size). Three weeks later the tumor had regained its original size. When this animal died after 4 months of observation, 2 large tumors were present, the second tumor having appeared 1 month before the death of the animal.

In animals No. 478 and No. 431, the original tumor was not palpable for 2 weeks and 2 months respectively. After the original tumor returned, 2 additional tumors appeared.

In 4 animals (Nos. 442, 457, 663, and 660) the original tumor had decreased in size when the animals died after an observation period of 2 to 3 months. This reduction in size was very marked in animal No. 457. The tumor was very small when the animal died after $2\frac{1}{2}$ months.

In animal No. 676, the original tumor remained small (no increase in size), yet 3 new tumors appeared in other parts of the body.

We have mentioned these individual variations in the growth and life cycle of these tumors in order to demonstrate different patterns of tumor growth. If we had a report of 500 controls, instead of 81, these individual groups would undoubtedly be represented by larger numbers.

We present in the accompanying illustrations and diagrams (Figs. 1–6) a number of interesting cases. The legends give a brief description of the life cycle of these tumors. Each of these figures presents a different and unusual pattern and demonstrates the numerous variations in the growth of these tumors.

Among the 81 controls, 13 were too putrefied to permit an autopsy. In 6 mice an examination was impossible due to cannibalism. Three animals were missing after 3 weeks, 2 and 4 months respectively, all with large tumors. The examination of the lungs among the remaining 59 controls which came to autopsy showed that 5 animals had adenomas, 25 had no tumors and 29 mice (49 per cent) presented metastatic carcinoma. It is of great interest to point out that among our autopsied healed tumor mice we have never seen a metastatic lung carcinoma.

Summary

In a previous paper we reported complete disappearance of 38 spontaneous mammary carcinomas in mice (strain A, Roscoe B. Jackson Memorial Laboratory) following intravenous treatment with spleen or yeast extracts. Biopsies had been performed in every case in order to establish beyond doubt the malignant nature of these tumors. The objection was raised that the successful treatment of these tumors was due to the biopsy and not to the treatment.

In order to meet this objection, 81 strain A mice which have a high incidence of spontaneous mammary carcinoma were subjected to biopsy and used as controls. No treatment was given. In none of these animals was a permanent and complete regression of the tumors observed. All control animals are dead, the vast majority with large and many with multiple tumors. Forty-nine per cent of the autopsied controls of the strain A mice presented metastatic carcinomas of the lungs.

We have never seen a metastatic lung carcinoma among the autopsied healed tumor mice.

Temporary regressions in the size of these tumors occur spontaneously. They are not necessarily due to any form of treatment.

It can be stated without reservation that a biopsy does not cause disappearance of these tumors.

REFERENCES

1. Lewisohn, R., C. Leuchtenberger, R. Leuchtenberger, and D. Laszlo. The Treatment of Spontaneous Breast Adenocarcinomas in Mice with Extracts of Spleen or Yeast. Am. J. Path., **17**: 251-260. 1941.

DESCRIPTION OF FIGURES 2 TO 6

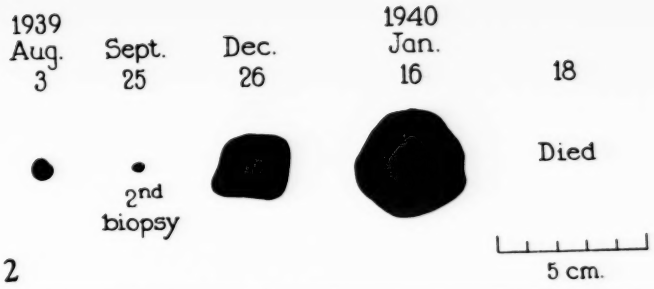
Fig. 2.—No. 237: Marked reduction in size of tumor after 1 month, second biopsy. Three months later large tumor.

Fig. 3.—No. 775: Original tumor has practically disappeared, but second tumor has grown to enormous size.

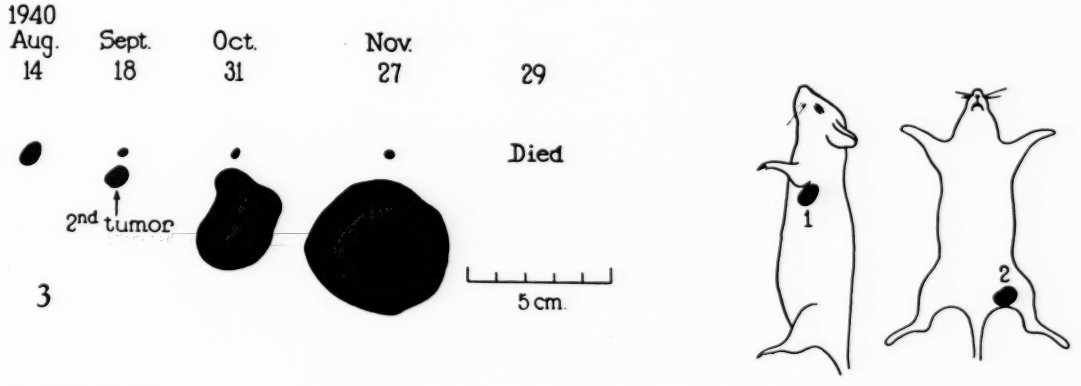
Fig. 4.—No. 478: Original tumor disappeared and returned. Two new tumors.

Fig. 5.—No. 431: Marked reduction in size of original tumor. Tumor not palpable for 2 months. Recurrence of original tumor and second tumor.

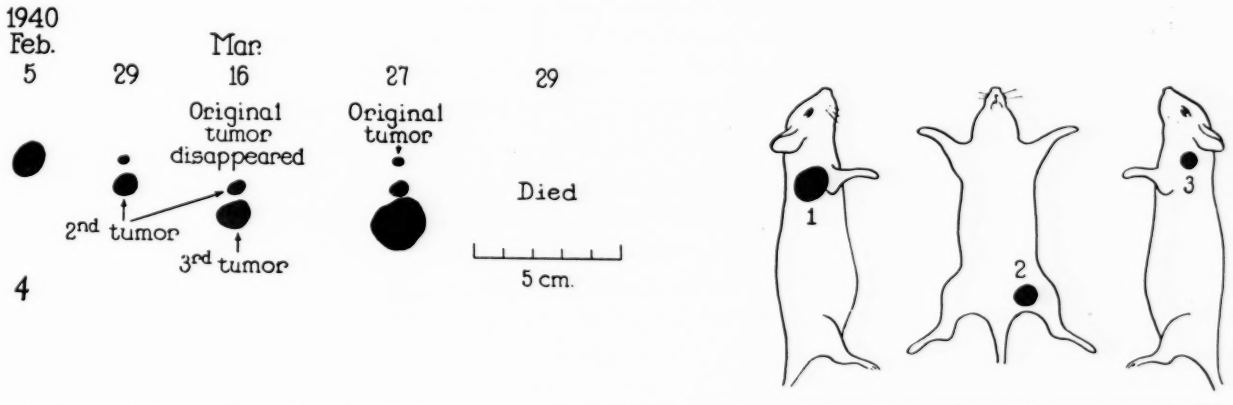
Fig. 6.—No. 273: Temporary regression with return to original size within 6 weeks. Three weeks later no tumor palpable. At time of death no palpatory evidence of tumor.



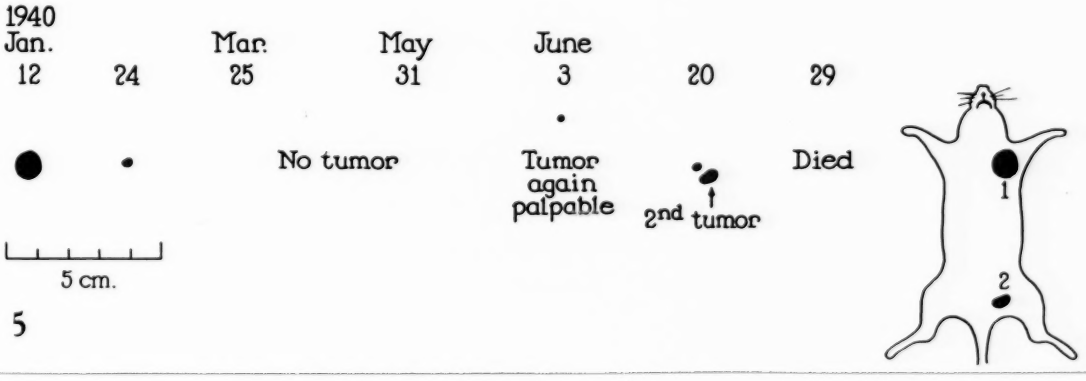
2



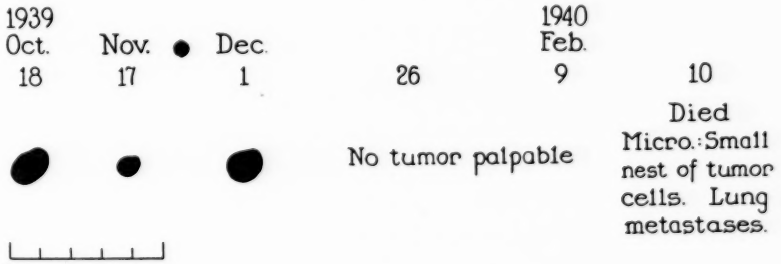
3



4



5



6

FIGS. 2-6.

Abstracts

Abstracts are grouped under the following main headings: Reports of Experimental Research, Comparative Oncology, Clinical and Pathological Reports, Statistics, and Cancer Control and Public Health. Subheadings are used in accordance with the subjects of papers abstracted.

The initials of the abstractor are placed at the end of each abstract. Contributors of abstracts published in this issue are: S. Bayne-Jones, M. Belkin, H. J. Creech, Amy DeBlasio, G. DeBlasio, Marie Duran-Reynals, W. U. Gardner, A. Haddow, E. L. Kennaway, E. A. Lawrence, W. V. Mayneord, A. Meltzer, F. L. Warren, and H. Gideon Wells.

REPORTS OF EXPERIMENTAL RESEARCH

Carcinogenic Compounds

BRUCE, W. F. [Cornell Univ., Ithaca, N. Y.] CARCINOGENIC HYDROCARBONS. V. A COMPARISON OF THE FLUORESCENCE INTENSITY OF CHOLANTHRENE AND CERTAIN OF ITS HOMOLOGS. J. Am. Chem. Soc., 63:304-305. 1941.

The previously described parallelism between carcinogenic activity and fluorescence intensity of C_{20}-homologs of cholanthrene in solutions of numerically equal concentrations was much less evident when comparisons were made using equimolecular concentrations. The photoelectric method utilizing several color filters may be of service to other investigators in various studies involving the use of aromatic hydrocarbons.—H. J. C.

CAMPBELL, J. A. [National Inst. for Med. Research, London] EFFECTS OF PRECIPITATED SILICA AND OF IRON OXIDE ON THE INCIDENCE OF PRIMARY LUNG TUMOURS IN MICE. Brit. M. J., 2:275-280. 1940.

In continuation of previous work mice were exposed to dust clouds "once an hour for 6 hours on five days of each week for a year."

Nature of dust	Number of mice living 10 months or longer	Mice with pulmonary tumors	
		Number	Per cent
Precipitated SiO_2	61	13	21.3
Controls	63	5	7.9
Brown oxide of iron, $Fe_2O_3 \cdot H_2O$.	52	17	32.7
Controls	52	5	9.6
$SiO_2 + Fe_2O_3 \cdot H_2O$	62	12	19.3
Controls	52	5	9.6
Nickel matte	47	14	29.8
Controls	64	8	12.5
Total of these and other experiments			
Dusted	727	..	31.5
Controls	593	..	11.3

In the dusted mice the proportion of lung tumors which are malignant is higher than in the controls; for details the tables in the original paper must be consulted. "The tracheo-bronchial lymph nodes of the mice exhibited some degree of fibrotic nodule formation due to silica. The dusting also caused an increase in the number of mice showing hypertrophy of the lymph tissue in the lungs and in the tracheo-bronchial lymph nodes. The connective tissue surrounding these nodes was also increased, as was the incidence of bronchopneumonia. The above results with silica and iron give experimental support to the statistical evidence that there is a relatively high incidence of carcinoma of the lung in metal-grinders, engineers, and foundry-workers." (References are given to the literature on this subject). "As regards other tumours, there were six cases of mammary tumours among 239 controls, compared with two cases among the 283 dusted mice."—E. L. K.

CASON, J., and L. F. FIESER. [Harvard Univ., Cambridge, Mass.] SYNTHESIS OF 4',8'-DIHYDROXY-1,2,5,6-DIBENZANTHRACENE AND ITS RELATION TO PRODUCTS OF METABOLISM OF THE HYDROCARBON. J. Am. Chem. Soc., 62:2681-2687. 1940.

Synthetic 4',8'-dihydroxy-1,2,5,6-dibenzanthracene (m.p. 415-418 corr. evac.) is not identical with the product of metabolism of the hydrocarbon in the rabbit (Levi and Boyland, Chem. and Indust., **15**:446. 1937) but it appears reasonably certain from absorption spectra data and mixed melting point determinations that it is identical with the compound excreted by mice and rats which have been injected with the hydrocarbon (Dobriner, Rhoads, and Lavin, Proc. Soc. Exper. Biol. & Med., **41**:67. 1939). It is postulated that a hydrocarbon on administration to an animal may be subjected to two independent and competing reactions proceeding by different mechanisms. The one responsible for tumor formation may be a chemical substitution at the reactive meso centers of the molecule whereas the other leading to detoxication may be a reaction of addition involving a different part of the molecule. The addition might take the form of "perhydroxylation" with subsequent change to the phenolic form or of combination with the disulfide bond of cystine to give a 1,2-addition product followed by loss of cysteine and acetylation, thus producing the mercapturic acid derivative of the hydrocarbon.—H. J. C.

CREECH, H. J. [Harvard Univ., Cambridge, Mass.] ISOCYANATES of 3,4-BENZPYRENE AND 10-METHYL-1,2-BENZANTHRACENE. J. Am. Chem. Soc., 63:576-578. 1941.

The above isocyanates were synthesized for further investigations of the carcinogenic and immunological properties of conjugates prepared from proteins and carcinogenic hydrocarbons. It is noted that 1,2,5,6-dibenzanthryl-9-

isocyanate appears to be somewhat more active carcinogenically than the parent hydrocarbon and much more active than the amine from which it is synthesized and to which it might be converted in the body by hydrolysis. This is significant because, with the exception of certain cyano compounds, the introduction of functional substituents into carcinogenic hydrocarbons invariably has led to a marked decrease in activity. Since it has been demonstrated that isocyanates can be readily combined experimentally with proteins, it is possible that the occurrence of some reaction of the described isocyanate with tissue proteins may be responsible for this effect. The new isocyanates are being tested for carcinogenic activity.—H. J. C.

CREECH, H. J., and R. N. JONES. [Harvard Univ., Boston, Mass.] THE CONJUGATION OF HORSE SERUM ALBUMIN WITH 1,2-BENZANTHRYL ISOCYANATES. J. Am. Chem. Soc., 62:1970-1975. 1940.

Conjugated proteins containing the 1,2-benzanthryl radical as the prosthetic group have been prepared for investigation of their possible carcinogenic and serological properties. Coupling was effected by interaction of the protein with the hydrocarbon isocyanates in an aqueous dioxane medium. The conjugates were purified by dialysis, centrifugation, filtration, and precipitation with ammonium sulfate and acetone. By ultraviolet spectrophotometry it has been possible to establish the fact that true conjugation has occurred and to determine the degree of conjugation. Purified samples of 1,2-benzanthryl-10-carbamido horse serum albumin contained 12 hydrocarbon residues per molecule of protein and it was found that 18 hydrocarbon residues had been introduced per molecule of protein in the case of the 1,2-benzanthryl-3-carbamido horse serum albumin without denaturing the protein component.—Authors' abstract.

FLORY, C. M. [Jersey City Med. Center, Jersey City, N. J.] THE PRODUCTION OF TUMORS BY TOBACCO TARS. Cancer Research, 1:262-276. 1941.

Twelve rabbits were painted on the ears with a tar produced by the destructive distillation of tobacco at from 350-700° C. Between the 49th and 79th day all rabbits developed tumors at the painting site. The rabbits lived from 238 to over 660 days. Sixty-eight of the tumors examined histologically were papillomas, and 5 were carcinomatoid tumors. No carcinomas were produced. A 120-350° C. destructive distillate tar produced tumors in 16 out of 17 rabbits, but more slowly than the 350-700° C. tar. Forty of these tumors were papillomas and 5 carcinomatoid tumors.

Twenty-four rabbits were painted with tar obtained by smoking tobacco in pipes. Tumors were produced in from 37 to 374 days in 22 out of the 24 rabbits. Seven animals lived over 640 days. Thirty-six tumors examined histologically were papillomas and 2 carcinomatoid tumors. No carcinomas were produced.

These tars produced tumors less readily when painted on mice, but the 350-700° C. tar (denicotinized) produced 8 papillomas and 1 squamous cell carcinoma which arose at 8.5 months when 14 mice were alive. The pipe tar (denicotinized) produced 2 papillomas and 1 squamous cell carcinoma, which arose at 17 months when 22 mice in this series were alive.—Author's abstract.

HAEREM, A. T. [Univ. of Chicago Sch. of Med., Chicago, Ill.] TISSUE RESPONSE to P-AMINO BENZENE SULFONAMIDE (SULFANILAMIDE) IN MICE. Proc. Soc. Exper. Biol. & Med., 45:536-539. 1940.

In experiments to test the possible carcinogenic action of sulfanilamide, 100 Swiss Albino mice were used. These mice were not "pedigreed" but "the natural incidence of cancer is low." Lard was used as the suspending agent for compounds tested. Experiments were made in 5 groups. (I) 20 mice were injected subcutaneously on 2 occasions with 15 mgm. 1,2,5,6-dibenzanthracene (Eastman Kodak Co.) in lard. Of these, 8 (40%) developed spindle cell sarcomas at the site of injection and died 150 to 200 days after the first injection. (II) 20 mice were injected subcutaneously with 15 mgm. sulfanilamide (Merck's) on 2 occasions. Of these, 2 (10%) developed tumors at the site of injection and died in 222 and 261 days. Six mice remained alive at the time of this report. The tumors were invasive spindle cell growths containing giant cells. One tumor invaded the muscles of the leg; the other eroded through the body wall and was adherent to the liver. No metastases were found. (III) 20 mice injected twice with 15 mgm. dibenzanthracene in lard were fed a diet containing 2.5% sulfanilamide. All died before 150 days. Two (10%) had spindle cell sarcomas at the site of injection. (IV) 20 mice fed a diet containing 2.5% sulfanilamide, without any injections, did not develop tumors. (V) 20 mice fed the regular diet did not develop tumors. No group was injected with lard alone.—S. B-J.

HIEGER, I. [Roy. Cancer Hosp. (Free), London] THE EXAMINATION OF TISSUES FOR CARCINOGENIC FACTORS. Science, 93:262-300. 1941.

Mammary tissue was removed from 7 dilute brown lactating does when their litters were almost weaned, was finely minced with scissors, shaken with distilled ether for about 20 minutes, stored in the refrigerator overnight, the ether changed, and the whole process repeated twice. The ether extracts were pooled, evaporated at low pressure and diluted with an equal volume of sterile olive oil. Thirty dilute brown female mice 6 to 8 weeks old, of commercial stock, received intraperitoneally 0.1 cc. of the olive oil solution per mouse per injection. Thirty control females of similar stock got 0.05 cc. sterile olive oil. Usually a fresh lot of mammary tissue was worked up for each series of injections. After 9 injections spread over 4 months, the does were mated. Of the 30 mice injected with the extract 14 littered; of the 29 controls 19 littered. The animals lived 25 months in all, but neither local reaction nor mammary carcinoma were observed.

In a second experiment each series consisted of 52 mice. The mammary tissue for each preparation was removed from 8 to 15 dilute brown lactating does and the injections were made subcutaneously. The injected does were of the same age and source as above. Eighteen sets of injections were spread over 5 months at roughly equal intervals. After the last injection, the animals were bred. Of the 51 mice receiving extract, 42 had litters; of 47 controls, 32 littered. At 22 months, mammary carcinomas had developed in 4 olive oil control mice (3 at the 19th and 1 at the 22nd month); while the series receiving tissue extract had developed similar tumors in 2 animals (17th and 21st month) and 1 spindle cell sarcoma in 1 animal

(19th month). The sarcoma is now growing in the second transplant generation. Hence an ether extract of dilute brown mammary tissue did not promote mammary cancer in female mice of ordinary mixed dilute brown stock.—M. B.

LAW, L. W. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] THE PRODUCTION OF TUMORS BY INJECTION OF A CAROCINOGEN INTO THE AMNIOTIC FLUID OF MICE. Science, 91:96-97. 1940.

Very small quantities of 1,2,5,6-dibenzanthracene injected into the amniotic fluid of mouse embryos have resulted in a greatly increased incidence of lung tumors among these animals at between 6 and 8 months of age.—Author's abstract.

LAW, L. W., and M. LEWISOHN. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] INDUCTION OF LYMPHOMATOSIS IN MICE FOLLOWING PAINTING WITH 9:10-DIMETHYL-1:2-BENZANTHRACENE. Proc. Soc. Exper. Biol. & Med., 43:143-146. 1940.

A general lymphomatosis occurs in mice of the dilute brown (Dba) strain (subline 212) following painting of the skin with a 0.3% solution of this carcinogen. External evidence of lymph gland abnormalities appear as early as 95 days after birth.—Authors' abstract.

MENKE, J. F. [Stanford Univ. Hosp., San Francisco, Calif.] A PRELIMINARY NOTE ON THE EXTRACTION OF A CARCINOGENIC FACTOR FROM PRIMARY HUMAN MAMMARY CANCER. Science, 92:290-291. 1940.

Two scirrhous carcinomas of the human breast were each treated as follows: The primary nodule with its immediately surrounding fatty tissue was dissected away, ground to a hash, covered with cold acetone, and stored in a refrigerator. The material was then extracted in a Soxhlet extractor at a low temperature with the following solvents in the order named (1) acetone, (2) ethyl ether, (3) petroleum ether (b.p. 35-60° C.), (4) absolute ethyl alcohol. Separate portions of the hash were then extracted with each solvent for 24 hours. The solvents were then removed by distillation at reduced pressure. Using the neutral fat as solvent, the fractions of each tumor were then combined, yielding 2 extracts.

Extract No. 1 was injected subcutaneously in the hind-breast region, into 4 virgin female mice, 1 month old, of the C57 black strain. Four injections of 0.2 cc. each were made over a period of 11 weeks. These mice were each allowed a single pregnancy. The first tumor, a spindle cell sarcoma, appeared 282 days after the first injection of this extract, at the site of the 1st injection. Transplants of this tumor into other mice of the same strain grew rapidly.

Extract No. 2 was injected into 5 virgin females, kept virgin throughout the experiment. Two injections were given, in the hindmost breast region, 17 days apart. A spindle cell sarcoma appeared 229 days after the 1st injection at the site of the 1st injection. Transplanted into other mice, the tumor grew rapidly. No other tumors appeared in either animal series.

Attempts are being made to isolate the active factor from the pooled extracts of several human cancers.—M. B.

MORTON, J. J. [Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.] THE PRESENT STATUS OF CARCINOGENS AND HORMONES IN CANCER RESEARCH. Surg., Gynec. & Obst. 72:345-362. 1941.

An exhaustive review citing 154 references, not suitable for abstracting but of great value to the investigator of cancer problems. The final conclusion is that "The process of cancerization still remains a mystery in its final steps."—H. G. W.

MOTTRAM, J. C. [Mount Vernon Hosp., Northwood, Middlesex, England] ABNORMAL PARAMECIA PRODUCED BY BLASTOGENIC AGENTS AND THEIR BEARING ON THE CANCER PROBLEM. Cancer Research, 1:313-323. 1941.

Paramecia were subjected to five blastogenic agents; hydrocarbons, gamma radiation, ultraviolet radiation, heat, and cold. Occasionally abnormal cells resulted which, on reproduction in the absence of the agents, produced populations of cells varying from apparently normal to very abnormal monstrous forms. Proneness to abnormality was transmitted rather than any special type of abnormality, and this transmission could be carried on indefinitely. These occurrences resembled in many respects the production of tumors in multicellular animals.

The first change in the *Paramecia* preceding the occurrence of abnormal cells was a change in the cytoplasm which interfered with their cleavage, leading first to delay, and later occasionally to a failure to cleave. After failure to cleave, the cell reorganized itself into a single cell having a double constitution. The subsequent irregular cell division of these doubles gave rise to the polymorphic abnormal populations resembling the cells of which tumors are composed.—Author's abstract.

POLAK, M. [Cancer Inst. of Paris and Inst. Nac. de Nutrición, Buenos Aires.] ACCIÓN DEL TABACO Y DE LA NICOTINA SOBRE DISTINTOS TEJIDOS CULTIVADOS "IN VITRO." [EFFECT OF TOBACCO AND NICOTINE ON TISSUE CULTURES.] Rev. Asoc. méd. argent., 54:31-32. 1940.

Experiments were carried out on cultures of different tissues from chicken and rat embryos, and from newborn rats. Weak dilutions of tobacco infusion exerted a favorable effect on the *in vitro* growth while dilutions more concentrated than 4% inhibited it. Nervous tissues were found more resistant than other tissues. Dilutions of nicotine have an analogous stimulating or inhibiting effect depending on their concentration.—M. D-R.

WOOD, J. L., and L. F. FIESER. [Harvard Univ., Cambridge, Mass.] SULFHYDRYL AND CYSTEINE DERIVATIVES OF 1,2-BENZANTHRACENE, 10-METHYL-1,2-BENZANTHRACENE AND 3,4-BENZPYRENE. J. Am. Chem. Soc., 62:2674-2681. 1940.

These types of compounds were prepared for investigation because of the physiological activity often associated with the mercapto group and because of the reported evidence for the formation in the organism of mercapturic acid derivatives of various aromatic hydrocarbons. As was the case with diazo coupling and lead tetraacetate oxidation, there was a rough parallelism between the carcinogenic activity of the above hydrocarbons and their reactivity towards substitution at reactive centers by sulfur monochloride. It is suggested that if an interaction of a carcinogenic hydrocarbon with cellular constituents represents a stage in the development of malignancy, it may be by the opening of protein disulfide linkages with attachment of the carcinogen to one sulfur and hydrogen to the other.—H. J. C.

Hormones

BITTNER, J. J. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] THE INFLUENCE OF ESTROGENS ON THE INCIDENCE OF TUMORS IN FOSTER NURSED MICE. Cancer Research, 1:290-292. 1941.

The administration of estrogenic hormones may result in the induction of mammary tumors in mice which have: (a) an inherited susceptibility for spontaneous tumors and an active milk influence; (b) an active milk influence without an inherited susceptibility for the development of spontaneous tumors. When the influence in the milk has been proved to be inactive few estrogenic tumors have been reported.—Author's summary.

BLAISDELL, J. S., W. U. GARDNER, and L. C. STRONG. [Yale Univ. Sch. of Med., New Haven, Conn.] ADRENAL GLANDS OF MICE FROM STRAINS WITH DIFFERENT SUSCEPTIBILITIES TO MAMMARY CANCER. Cancer Research, 1:283-289. 1941.

The adrenal glands from 130 male and female mice from 11 different inbred strains which showed varying susceptibilities to mammary tumors were studied. In 6 strains mammary tumors rarely or never developed spontaneously. Two types of adrenal degenerative processes, "brown degeneration" and amyloid degeneration, were observed in 70.7% and 31.3%, respectively, of the females. The incidence of these degenerative changes varied greatly among the different strains. No consistent relationship existed between the frequency of either of the two degenerative changes and the potentiality of mice of the strains to develop mammary cancer. Also the extent of involvement of the adrenal cortex could not be correlated with any tendency for mammary tumors. The two degenerative processes could not be considered as immediate or remote causes for the development of mammary cancer. A common etiological factor responsible for the development of brown degeneration and mammary tumors was not evident. Four photomicrographs and 3 charts illustrate the paper.—Authors' summary.

DOBROVOLSKAÏA-ZAVADSKAÏA, N., and P. ZEPHIROFF. [Inst. du radium de l'Univ. de Paris] EFFET DES PRODUITS D'ORIGINE ÉPIPHYSAIRE ET D'ORIGINE HÉPATIQUE SUR LA CROISSANCE DES TUMEURS CHEZ LA SOURIS. [EFFECTS OF PRODUCTS OF THE PINEAL GLAND AND LIVER ON THE GROWTH OF TUMORS IN MICE.] Compt. rend. Soc. de biol., 134:60-63. 1940.

A series of 14 tumor-bearing mice were treated successively with 3 preparations: a) "Antisexual" substance isolated from the urine of a 4-year-old girl, 1 cc. corresponding to 100 cc. of urine, in daily injections of 0.025 to 0.05 cc.; b) aqueous solution of a commercial pineal extract, 1 cc. equivalent to 0.02 gm. of the fresh organ, in daily injections of 0.05 to 0.1 cc.; c) a liver extract in oil, 1 cc. corresponding to 10 gm. of fresh organ, in daily doses of 0.05 cc. given subcutaneously. The transition from one mode of treatment to the other was often marked by a sudden change in tumor growth rate, but the direction of the change was not always the same for all animals; sometimes it was greater, sometimes less. In spite of individual variation, it may be stated that the greatest retardation of tumor growth followed the urine extract.—Authors' abstract.

DOBROVOLSKAÏA-ZAVADSKAÏA, N., and P. ZEPHIROFF. [Inst. du radium de l'Univ. de Paris] ÉTUDE QUANTITATIVE DE LA CROISSANCE DES TUMEURS CHEZ LES SOURIS SOUMISES A L'ACTION D'EXTRAITS HEPATIQUE ET CORTICO-SURRÉNALIEN. [EFFECTS OF EXTRACTS OF LIVER AND ADRENAL GLAND ON THE GROWTH OF TUMORS IN MICE.] Compt. rend. Soc. de biol., 133:216-218. 1940.

In this paper are recorded results obtained on 16 tumor-bearing mice divided in 3 groups: 1) those treated with liver extract only; 2) those treated with adrenal gland extract only; and 3) those treated first with adrenal extract alone, and then with adrenal and liver extracts combined. A marked individual variety of response was noted. The mean survival in the 3 groups was 31, 38, and 89 days, respectively.—Authors' abstract.

DOBROVOLSKAÏA-ZAVADSKAÏA, N., and P. ZEPHIROFF. [Inst. du radium de l'Univ. de Paris] PEUT-ON INFLUENCER L'EVOLUTION DES TUMEURS DE SOURIS PAR DES EXTRAITS ÉPIPHYSAIRE ET HEPATIQUE? [EFFECTS OF EXTRACTS OF THE PINEAL GLAND AND LIVER ON THE DEVELOPMENT OF TUMORS IN MICE.] Compt. rend. Soc. de biol., 134:79-81. 1940.

In 17 mice tumor growth was affected by extracts of pineal and liver, but the effect was variable, apparently depending upon the physiological state of the animal and the chemico-histo-pathological characteristics of the tumor.—Authors' abstract.

ZEPHIROFF, P., C. DROSDOVSKY, AND N. DOBROVOLSKAÏA-ZAVADSKAÏA. [Inst. du radium de l'univ. de Paris] PRÉSENCE D'UNE SUBSTANCE OESTROGÈNE DANS LES OVAIRES DES ANIMAUX IMPUBÈRES (VEAUX). [OCCURENCE OF ESTROGENIC SUBSTANCE IN THE OVARIES OF IMMATURE CALVES.] Compt. rend. Soc. de biol., 133:236-238. 1940.

In view of the established influence of folliculin on the origin of mammary cancer in mice, its presence in infantile ovaries was investigated.

Calf ovaries, weighing about 2.25 gm. each (the average weight of a cow ovary is 12 gm.), were pulped, dried at low temperature, and extracted with alcohol and ether. After distillation and purification, the product was dissolved in papaver oil. One cc. of this solution corresponded to 40 gm. of fresh ovary.

Tests of this preparation on ovariectomized rats by the Allen and Doisy method showed that the quantity of folliculin in infantile ovaries approximates that in adult ovaries.—Authors' abstract.

FRIEDRICH-FREKSA, H. [Kaiser-Wilhelm-Inst. f. Biochemie, Berlin-Dahlem] SEXUALHORMONE UND ENTSTEHUNG BÖSARTIGER GESCHWÜLSTE. [SEX HOMONES AND THEIR RELATION TO MALIGNANT TUMORS.] Berichte ges. Gynäk. u. Geburtshilfe, 40:225-249. 1940.

A well arranged, concise, and critical review of the reported investigations on the possible role of estrogens in carcinogenesis. The observations on the effect of estrogens on connective tissues, skin, lymphoid tissues, mammary glands, uteri, and endocrine glands in the various species of laboratory animals are summarized. The author concluded (1) that estrogens could induce regularly only mammary tumors in mice from strains in which the females showed a tendency to give rise to such tumors spontaneously. The different synthetic and natural estrogens which resulted in mammary growth were all carcinogenic under such conditions, revealing no chemical specificity as shown by the carcinogenic hydrocarbons.

Larger doses may be followed by a few tumors in rats and in mice of low tumor strains. (2) The clinical use of estrogens in therapeutic doses is not carcinogenic.—W. U. G.

LIPSCHÜTZ, A., and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] STRUCTURE AND ORIGIN OF UTERINE AND EXTRAGENITAL FIBROIDS INDUCED EXPERIMENTALLY IN THE GUINEA PIG BY PROLONGED ADMINISTRATION OF ESTROGENS. Cancer Research, 1:236-249. 1941.

Fibroid tumors of the uterus and of extra-uterine tissues in the abdominal cavity were produced experimentally in guinea pigs by prolonged administration of estrogens. The structure of these tumors is described and illustrated in 5 plates including 5 photographs of the gross tumors and 37 photomicrographs of sections.—S. B-J.

NOBLE, R. L., and J. B. COLLIP. [McGill Univ., Montreal] REGRESSION OF OESTROGEN INDUCED MAMMARY TUMOURS IN FEMALE RATS FOLLOWING REMOVAL OF THE STIMULUS. Canad. M. A. J., 44:1-5. 1941.

The observations on estrogen-treated female rats described in an earlier paper (Canad. M. A. J., **42**:413-417. 1940) were extended over a more prolonged period and certain characteristics of the mammary tumors arising from the treatment studied. The mammary tumors grow slowly and continuously, provided the estrone pellet is still palpable, until the death of the animal. The rat may live 9 months after the first tumor appears. Over such periods new tumors occur in other mammae until the mammary area is a mass of tumors. In one case the tumors weighed 134 gm. at autopsy. In 5 cases homotransplantation was unsuccessfully attempted into female rats of the same strain previously implanted with estrone tablets. In 7 cases biopsy was performed on a tumor and a piece of it grafted subcutaneously in the mid-line of the same animal's back. In 2 animals growth of the transplant occurred. Four rats with actively growing tumors received subcutaneous injections of 2.5 mgm. of progesterone daily for 13 to 20 days. In every case the tumors stopped growing and in 3 instances regressed to less than one-half their original size. On cessation of progesterone treatment the tumors resumed their growth. The estrone pellets were removed from 4 rats bearing rapidly growing multiple tumors (in one case typical infiltration of the surrounding tissue by tumor cells was present and this animal also bore a successful homotransplant). Following the removal of the pellets all the tumors stopped growing and regressed rapidly. After 3 weeks only slight residual thickening of the mammary tissue was palpable. Two chromophobe adenomas of the pituitary were noted during the first year of treatment; with more prolonged treatment nearly all animals exhibited these tumors. In preliminary experiments mammary tumors have been obtained after implantation of pellets at 28 days of age. Tumors were also found in male rats and after implantation of estradiol tablets.—F. L. W.

NOBLE, R. L., C. S. McEUEN, and J. B. COLLIP. [McGill Univ., Montreal] MAMMARY TUMOURS PRODUCED IN RATS BY THE ACTION OF OESTRONE TABLETS. Canad. M. A. J., 42:413-417. 1940.

Estrone tablets varying from 1 to 7 mgm. in weight were implanted either singly or in groups, in the subcutaneous tissue of the back of 5- to 7-day-old female rats of the black-hooded strain. One to three tablets containing 4 to 11 mgm. estrone were used for each rat. The animals were examined by palpation at regular intervals and a biopsy performed when any palpable mass reached 1 cm. diameter. At the end of 8 months 47 animals were surviving and retained pellets. Tumors of the mammary gland were found in 28 of 49 rats. The first tumor was palpated on the 226th day. The average time of appearance of the tumors was not affected by the total dose of estrone employed. The tumors were frequently multiple, slow growing, and not near the site of implantation of the tablet. The glandular tissue in the mammae of all the animals examined was increased in amount and the ducts showed proliferative changes and were usually distended with secretion. In some cases large cystic areas were seen. The tumors formed compact, clearly defined areas of cells, in which the epithelial components showed an extremely marked hyperplasia. The masses of cells formed large, rounded, sharply demarcated solid areas, but in some a small central lumen was present into which the innermost cells were shed. Intense epithelial proliferation and squamous cell metaplasia were present, in some cases associated with an overgrowth of fibrous stroma. In spite of the extreme hyperplasia the cells showed no tendency to invade the stroma or to penetrate the well-defined basement membrane or adjacent tissues. Other effects usually associated with prolonged estrogen therapy were noted. These included enlargement of the pituitary gland with adenoma formation, ovarian atrophy, uterine fibrosis with squamous cell metaplasia, and inhibition of body growth. Subcutaneous injection of thyrotropic hormone had no effect on the incidence of mammary tumors.—F. L. W.

SAXTON, J. A., JR. [Cornell Univ. Med. Coll., New York, N. Y.] THE RELATION OF AGE TO THE OCCURRENCE OF ADENOMA-LIKE LESIONS IN THE RAT HYPOPHYSIS AND TO THEIR GROWTH AFTER TRANSPLANTATION. Cancer Research, 1:277-282. 1941.

Chromophobe adenoma-like lesions were observed in the hypophyses of 17 out of 43 rats of the Yale strain aged between 1 and 2 years. None was found in 84 rats of less than 1 year of age. The lesions were frequently multicentric in origin. Bioassay of adenoma-like tissue failed to indicate the presence of gonadotropic, thyrotropic, or adrenotropic hormones, whereas these hormones were demonstrated in normal rat hypophyseal tissue. Growth of homologous intra-ocular transplants of adenoma-like tissue occurred in 3 out of 9 instances after latent periods of 7, 10, and 12 months, at a time when the recipients had reached an advanced age. The advanced age at which adenoma-like lesions appear in the hypophysis, their multicentric origin, and the prolonged latent periods of the transplants suggest that their growth is dependent upon factors of the host which make their appearance at an advanced age.—Author's abstract.

SNELL, G. D., E. FEKETE, K. P. HUMMEL, and L. W. LAW. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] THE RELATION OF MATING, OVULATION AND THE ESTROUS SMEAR IN THE HOUSE MOUSE TO TIME OF DAY. Anat. Rec., 76:39-54. 1940.

Estrus usually occurs between 10 P.M. and 1 A.M.; ovulation between midnight and 2 to 3 A.M. Ovulation is

unaffected by mating. The duration of estrus probably does not exceed 24 hours. By the use of electric lights it was possible to condition the mice to artificial day and night periods to which they responded.—Authors' abstract.

SPIEGEL, A. [Zoolog. Inst. und Patholog. Inst., Friedrich-Schiller-Univ. Jena] ÜBER DAS AUFTRETEN VON GESCHWÜLSTEN DER NEBENNIERENRINDE MIT VERMÄNNLICHENDER WIRKUNG BEI FRUHKASTRIERTEN MEERSCHWEINCHENMÄNNCHEN. [THE APPEARANCE OF TUMORS OF THE ADRENAL CORTEX WITH MASCULINIZATION OF MALE GUINEA PIGS CASTRATED AT EARLY AGE.] Virchows Arch. f. path. Anat., 305:367-393. 1939-40.

Tumors of adrenal cortical tissue were found in 3 castrated male guinea pigs 2½ to 4 years old. These animals had been castrated when 1 to 3 days of age. The tumors varied in size and were composed of large lipoid-containing cells arranged in cords and separated by delicate connective tissue septa. Cells containing an alcohol-insoluble brown pigment were found in some tumors and in the zona reticularis. Two of the castrated males had well developed accessory genital tissues namely, penis, prostate, and seminal vesicles. One male copulated with an estrous female. The genital tissues of the third resembled those of young males. Similar tumors were not observed in 6 males and 5 female guinea pigs from 3 to 6 years of age and castrated when very young. The literature on the effect of castration on the adrenal glands, the appearance of adrenal tumors in laboratory animals and on the urinary assays for androgenic substances in urine from cases of genito-adrenal conditions in man are reviewed. Eleven microphotographs are included.—W. U. G.

SPIEGEL, A. [Zoolog. Inst. und Patholog. Inst., Friedrich-Schiller-Univ., Jena] AUFTRETEN VON ADENOMEN DER NEBENNIERENRINDE MIT VERMÄNNLICHENDER WIRKUNG BEI FRÜHKASTRIESTEN MEERSCHWEINSCHENMÄNNCHEN. [APPEARANCE OF ADENOMAS OF THE ADRENAL CORTEX WITH MASCULINIZATION OF MALE GUINEA PIGS CASTRATED AT EARLY AGES.] Klin. Wchnschr., 18:1068-1069. 1939.

Same material described in another abstract.—W. U. G.

WOLFF, J. K., L. F. FIESER, and H. B. FRIEDGOOD. [Harvard Univ., Cambridge, and Peter Bent Brigham Hosp., Boston, Mass.] NATURE OF THE ANDROGENS IN FEMALE ADRENAL TUMOR URINE. J. Am. Chem. Soc., 63:582-593. 1941.

A detailed account is given of the application of efficient and specific methods for the determination of certain urinary steroids. The processes of hydrolysis of the urine, extraction and separation of the various fractions, isolation and identification of the steroids by chemical means and by polarographic analyses are described thoroughly. The androgenic fraction from the acid-hydrolyzed urine of a girl with a recurrent corticoadrenal tumor was found to contain an abnormally high content of 3α-hydroxyaetiocholanone-17 (10-fold) and dehydroisoandrosterone (100-fold). Part of the latter compound underwent transformation into 3-chloro-Δ^5-androstenone-17 during the acid hydrolysis. Two other steroids ($\Delta^{3,5}$-androstadienone-17 and 3α-hydroxyandrostenone-17) not present in normal urine were also isolated. The latter compound is a new steroid (m.p. 181-183° corr., $[\alpha]_D^{26.5} + 122$ in alcohol) which has been assigned the mentioned tentative structure in which the only point of uncertainty is the exact location of the double bond. The estrogen and androsterone levels of this pathological urine were normal.

It is suggested that a possible carcinogen conceivably might be formed in the adrenal gland by the condensation of dehydrocorticosterone at the activated positions 12 and 21 with a carbonyl compound, possibly an easily degraded derivative of one of the ketols of the adrenal cortex, to give a new six-membered ring. Elimination of oxygen followed by dehydrogenation could lead to the formation of a hydrocarbon possessing the cholanthrene structure. Evidence is presented for the occurrence in the adrenal gland of reactions of the latter two types.—H. J. C.

ZEPHIROFF, P., AND N. DOBROVOLSKAÏA-ZAVADSKAÏA. [Inst. du radium de l'Univ. de Paris] QUELQUES DONNÉES SUPPLÉMENTAIRES CONCERNANT LA SUBSTANCE ANTISEXUELLE ISOLÉE DE L'URINE D'UNE FILLETTE DE QUATRE ANS. [ANTI-ESTRUS SUBSTANCE ISOLATED FROM URINE OF A FOUR-YEAR-OLD-FEMALE.] Compt. rend. Soc. de biol., 134:77-78. 1940.

The above-mentioned substance from the urine of a 4-year-old girl (1 cc. of aqueous solution corresponding to 50 cc. of urine) was examined: A. In relation to the gonadotropic hormone (prolan) isolated from the urine of a pregnant woman, 1 cc. of the solution used corresponding to 20 cc. of urine. Of 3 groups of young rats, 3 males and 3 females in each group, one group served as controls. The other two were injected daily for 5 days, one group with 0.5 cc. of the prolan solution prepared from the pregnancy urine, the other with 0.5 cc. of this prolan solution plus 0.5 cc. of the preparation from the child's urine. On the 6th day, all animals were sacrificed and their genital organs compared. Advanced development was found in the second group only; *i.e.*, that receiving the prolan. B. In relation to the estrogenic hormone, two groups of 5 ovariectomized female rats were used. The first group received, during 2 days, 3 injections of 5 rat units each of folliculin. The second group received similar injections plus 3 injections of the extract from the child's urine (0.5 cc.). Typical estrus appeared in all animals on the 3rd day. Thus we may conclude that the substance from the child's urine was able to neutralize the gonadotropic hormone, but not the estrogenic effect of the folliculin.—Authors' abstract.

Genetics

MURRAY, W. S., and J. G. HOFFMAN. [New York State Inst. for Study of Malignant Diseases, Buffalo, N. Y.] PHYSIOLOGICAL AGE AS A BASIS FOR THE COMPARISON OF STRAINS OF MICE SUBJECT TO SPONTANEOUS MAMMARY CARCINOMA. Cancer Research, 1:298-307. 1941.

A survey of the methods used in computing the incidence of mammary cancer is presented. These methods are all applied to data accumulated on the dilute brown, Bittner albino, and Marsh albino breeding females. The results of these computations are compared with those obtained by the use of a proposed method which combines a distribution of these same data according to physiological or equivalent age and the principle of corrected rates.—Authors' abstract.

Radiation

GOLDFEDER, A. [New York Univ. Med. Coll., New York, N. Y.] FURTHER STUDIES ON THE EFFECTS OF IRRADIATION ON PROLIFERATION AND METABOLIC PROCESSES OF NORMAL AND MALIGNANT TISSUES. IV. EFFECTS PRODUCED BY DIFFERENT DOSAGE RATES OF X-RAY RADIATION ON THE PROLIFERATION OF VARIOUS TISSUES GROWN IN VITRO. Radiology, 35:210-228. 1940.

The experimental methods and results of x-ray irradiation of 24- to 48-hour-old rat kidney and spleen, sarcoma 180 from 8- to 10-day-old growing tumors, and 8-day-old chick embryo hearts with varying dosage rates are presented. The freshly excised tissue was implanted into a nutritive medium, irradiated, and then transferred to a fresh medium. The effect of irradiation was evaluated by comparing the new growth in the irradiated explants with that in the nonirradiated controls. Three different x-ray dosage rates were used: 234 r/min., 936 r/min. and 10,000 r/min. The critical dose (the single, massive dose preventing proliferation) for rat kidney treated at the rate of 936 r/min. is 90,000 to 95,000. When treated at the rate of 234 r/min., it is 75,000 to 80,000 r; and at the rate of 10,000 r/min., it is 130,000 to 140,000. It is noted that the effects of irradiation depend upon the dosage rate and time of exposure. Similar results were obtained with the other tissues. When chick embryo heart is irradiated at the rate of 936 r/min., the critical dose is 140,000 to 150,000 r; while at 10,000 r/min. it is 250,000 to 260,000 r. At 936 r/min. the critical dose for both rat spleen and sarcoma 180 is 50,000 to 60,000 r. For sarcoma 180 at 10,000 r/min. it is 85,000 to 90,000 r.

It is speculated that the destructive effects of radiation may be effective during only a relatively short period in the life cycle of the cell, and that a lower dosage rate with a correspondingly longer period of exposure would increase the probability of direct hits during these sensitive periods, thereby decreasing the total dosage for a given biological effect.—E. A. L.

GRAY, L. H., J. C. MOTTRAM, J. READ, and F. G. SPEAR. [Mount Vernon Hosp., Northwood, Middlesex, and Strangeways Lab., Cambridge, England] SOME EXPERIMENTS UPON THE BIOLOGICAL EFFECTS OF FAST NEUTRONS. Brit. J. Radiol., 13:371-388. 1940.

A quantitative comparison has been made of the action of fast neutrons (2.8 MEV) and gamma rays in inhibiting mitosis in tissue cultures of chick embryo fibroblasts and in the root of the broad bean. The method of dose measurement used makes it possible to compare the effects of equal amounts of gamma ray and neutron energy absorbed per unit volume of tissue. In the case of both tissue cultures and bean roots, the curve relating residual mitosis and dose was found to be approximately exponential, and in the former case the shape of the curve was significantly different from that of the corresponding gamma ray curve, which is of the usual sigmoid type. No intensity dependence was observed. The exponential nature of the neutron curve suggests that the inhibition of mitosis is brought about by one, or at most a few, recoil protons, and the magnitude of the neutron dose which reduces mitosis to 37% then shows that the volumes within which these particles must pass to be effective, if spherical, have radii of about 2μ or 3μ respectively for the 2 tissues studied. The results indicate that in each case the same number of ions have to be produced within these volumes whichever radiation is used. The difference between both shape and magnitude of the mitosis dose curves for gamma rays and neutrons is then accounted for quantitatively by the fact that in the former case the ionization would be produced by several hundred secondary electrons, and in the latter by a single recoil proton.

Other quantitative studies of the biological effects of fast neutrons are reviewed, and an hypothesis is advanced to account for the large relative efficiency of neutron compared with gamma ray energy which has been observed in some cases. The hypothesis is elaborated in some detail for the case of the lethal action of neutrons on broad bean roots ($\gamma/N=11$), and it is shown that both the high efficiency and the shape of the mortality curve may be accounted for by simple and plausible assumptions.—W. V. M.

MARSHAK, A. [Univ. of California, Berkeley, Calif.] UPTAKE OF RADIOACTIVE PHOSPHORUS BY NUCLEI OF LIVER AND TUMORS. Science, 92:460-461. 1940.

Mice carrying 10 to 15-day lymphoma transplants were injected intravenously with 0.1 cc. of isotomic Na_2HPo_4 containing 6 to 10 microcuries of P_{32}. The livers were cleared of blood by perfusion with saline, immersed in ice-cold 5% citric acid for ½ hour, and the nuclei isolated by centrifugation of the pulped tissues. Tumors were removed from the same animals and treated similarly to obtain nuclei. Hemocytometer counts showed negligible contamination by fragments of cytoplasm. Although the fraction of the P_{32} bound by the liver nuclei remains constant at 0.3-0.4 of the P_{32} activity of the whole tissue, the relative concentration in the tumor nuclei rises to more than twice that in the tumor tissue. Similar results have been obtained with sarcoma 180 in the mouse. The uptake of P_{32} cannot be attributed to simple chemical exchange since isolated nuclei *in vitro* take up much less P_{32} than *in vivo*. Experiments with normal and regenerating rat liver tissue and rat carcinoma 256 indicated that the greater concentration of P_{32} by tumor nuclei is not characteristic of the tumor *per se* but should be attributed to greater mitotic activity.—M. B.

RUSCH, H. P., B. E. KLINE, and C. A. BAUMANN. [Univ. of Wisconsin, Madison, Wis.] CARCINOGENESIS BY ULTRAVIOLET RAYS WITH REFERENCE TO WAVE LENGTH AND ENERGY. Arch. Path., 31:135-146. 1941.

The carcinogenic wave lengths of the spectrum were found to lie between 2,900 and 3,341 angstroms. Wave lengths of 2,537 or 3,341 angstroms and above were noncarcinogenic. The carcinogenic wave lengths thus coincided in part with those most potent in the production of erythema, but radiant energy with a wave length of 2,537 angstroms produced erythema without inducing tumors in mice. Very little radiant energy was needed to initiate those changes which culminate in tumor formation. The minimum time for the development of tumors appeared to be about 2½ months, and it was not necessary to irradiate the animals throughout the precancerous period. Once initiated, carcinogenesis proceeded without further

exposure, and sometimes several months elapsed between the end of irradiation and the appearance of tumors.—H. G. W.

ZAHL, P. A., and F. S. COOPER. [Memorial Hosp. and Haskins Labs., New York, N. Y.] LOCALIZATION OF LITHIUM IN TUMOR TISSUE AS A BASIS FOR SLOW NEUTRON THERAPY. Science, 93:64-65. 1941.

The passage of slow neutrons through body tissues results in little or no damage. However, if these slow neutrons are introduced into a tissue which contains boron or lithium or their compounds, nuclear capture reactions occur causing local destruction of tissue. Lithium salts of pontamine sky blue 6B, trypan blue, and carminic acid, dyes which are selectively localized in malignant tissue, were injected intravenously into mice bearing spontaneous mammary tumors and mice bearing implanted tumors of sarcoma 180. After suitable periods, animals were sacrificed and the tumor and other tissues analyzed spectroscopically for lithium. A maximum gain of about 43% in the radiation dosage of the tumor over that of other tissues in the same animal was attained. The optimum treatment condition is determined by the difference in energy between tumor and healthy tissue; this difference should be as large as possible.—M. B.

Biochemistry and Nutrition—Chemotherapy

BEHRENS, O. K., F. LIPMANN, M. COHN, and D. BURK. [Cornell Univ. Med. Coll., New York, N. Y., and Nat. Cancer Inst., Bethesda, Md.] THE NONSPECIFICITY OF AMINO-ACID CONFIGURATION IN MALIGNANT TISSUE HYDROLYSATES. Science, 92:32-34. 1940.

Samples of human normal liver and liver carcinoma showed upon analysis with deuterium approximately equal quantities of d(−)glutamic acid of the order of several tenths % of the original dry weight of tissue. These results agree in order of magnitude with the majority of values reported by others for normal or malignant tissues by various methods. The most conclusive evidence against the specificity of d(−)glutamic acid in malignancy is that l(−)glutamic acid is racemized in hot HCl. The rates are of an order sufficient to account for essentially all the isolated or analyzed quantities of d(−)glutamic acid reported in hydrolysates of protein, normal or tumor tissue. The view that cancer tissue but not normal tissue or protein is composed of partly unsaturated d(−)amino acids is no longer held tenable, both as regards glutamic acid as well at total d(−)amino acid. The relation of the authors' investigations to those of others in this field is discussed.—M. B.

BOWMAN, R. O., and H. R. MOTTSHAW. [Rhode Island Hosp. Lab. and Brown Univ., Providence, R. I.] FAILURE TO FIND CARCINOGENS IN URINE FROM PATIENTS WITH CANCER. Cancer Research, 1:308-309. 1941.

Urine from cases of proved human cancer was shown to contain no substance that would produce tumors in C57 black mice. Proteins (? virus) could not be salted out. Growth stimulating factor (Rohdenburg and Nagy) was toxic but did not produce tumors. Benzene and then ether extracts of dehydrated urine, when used for tarring or injection, were also negative.—Authors' abstract.

CHRISTIANI, A. KARZINOLYSE. [CARCINOLYSIS.] Deutsche med. Wchnschr, 48:1335-1336. 1940.

The author's theory is: Normal serum contains a carcinolytic enzyme. This is inhibited by a substance in cancer serum. The latter is cholesterinbutyrate, which is synthesized only in small quantities in the cancer patient. The lytic agent is an oxidation product of "ergosterin," traces of which normally accompany cholesterol. This product deprives cholesterinbutyrate of its protective properties. This oxidation product of ergosterin is only produced in the normal patient and requires the presence of oxidases. These are lacking in cancer patients. (Radium radiation, however, has a strong oxidative action capable of producing this lytic ferment in the cancer patient.) The normal patient does not produce cholesterinbutyrate because of the action of dehydrase which alters the butyric acid to produce an inactive ester. In cancer patients dehydrase plays no role; cholestrol and unaltered butyric acid combine.—A. M.

DOBROVOLSKAÏA-ZAVADSKAÏA, N., and P. ZEPHIROFF. [Inst. du radium de l'Univ. de Paris] L'EFFET D'UN TRAITEMENT COMBINÉ PAR LA CORTINE HYDROSOLUBLE, ET PAR LA CYSTÉINE CHEZ UNE SOURIS ATTEINTE D'ADÉNOCARCINOME DE LA MAMELLE. [EFFECT OF CORTIN AND CYSTEINE ON ADENOCARCINOMA OF THE BREAST IN MICE.] Compt. rend. Soc. de biol., 133:391-394. 1940.

In one mouse of strain XLIII treated with water-soluble cortin, a striking increase of tumor growth was obtained by subcutaneous injections of cysteine (3 injections of 0.1 cc. each of a 2% solution and a 4th injection of 0.1 cc. of 1% solution). Coincidentally with these injections, 2 tumors which had remained stationary during a month's treatment with cortin, began to increase progressively, and many new tumors appeared. Finally this mouse died with 9 tumors, which was quite unusual. Such a multiplicity of tumors has been observed only once, and then in strain XIX, in which multiple tumors proved to be hereditary. The treatment with cortin was not interrupted. It did not stop the growth of tumors, but perhaps retarded their development slightly and thus determined the long survival of this animal (103 days).—Authors' abstract.

KENNEY, J. M. [MEMORIAL HOSP., New York, N. Y.] THE METABOLISM OF RADIOACTIVE PHOSPHORUS BY MALIGNANT NEOPLASMS IN HUMAN BEINGS. Surgery, 9:327-328. 1941.

Patients with lymphoma, inoperable cancer, and osteogenic sarcoma were given tracer doses of radioactive P. Biopsies were performed after 5 to 7 days. The percentage of retained P was determined and translated into terms of equivalent roentgens. As high as 1000-1500 equivalent r may be found in the nodes of lymphosarcoma. Osteogenic sarcoma absorbed more than any tissue. A tumor dose of 3,000-4,000 equivalent r may be possible, but the effects on the patient are not known.—A. M.

LEWISOHN, R., C. LEUCHTENBERGER, R. LEUCHTENBERGER, and D. LASZLO. [Mt. Sinai Hosp., New York, N. Y.] THE FATE OF SPONTANEOUS MAMMARY CARCINOMAS IN MICE AFTER SIMPLE BIOPSY. Cancer Research, 1:324-327. 1941.

In a previous paper the authors reported complete disappearance of 38 spontaneous breast carcinomas in mice (strain A, Roscoe B. Jackson Memorial Laboratory) fol-

lowing intravenous treatment with spleen or yeast extracts. Biopsies had been performed in every case in order to establish the malignant nature of these tumors beyond doubt. The objection was raised that the successful treatment of these tumors was due to the biopsy and not to the treatment. In order to meet this objection, 81 strain A mice with a high incidence of spontaneous mammary carcinoma were subjected to biopsy and used as controls. No treatment was given. In none of these animals was a permanent and complete regression of the tumors observed. All control animals are dead, the vast majority with large and many with multiple tumors. Forty-nine per cent of the autopsied controls of strain A presented metastatic carcinomas of the lungs. The authors have never seen a metastatic lung carcinoma among the autopsied healed tumor mice. Temporary regressions in the size of these tumors occur spontaneously. They are not necessarily due to any form of treatment. It can be stated without reservation that a biopsy does not cause disappearance of these tumors.—Authors' abstract.

LEWISOHN, R., C. LEUCHTENBERGER, R. LEUCHTENBERGER, and D. LASZLO. [Mt. Sinai Hosp., New York, N. Y.] THE TREATMENT OF SPONTANEOUS BREAST ADENOCARCINOMAS IN MICE WITH SPLEEN OR YEAST EXTRACT. Am. J. Path., 17:251-260. 1941.

This paper presents further details of technical improvements in the spleen and yeast extracts and a report of 38 out of 189 (20%) spontaneous breast cancers in mice which regressed following intravenous injections of these extracts. This reduction from the 30% in which the treatment is usually successful is to be explained by a severe intercurrent infection which killed many of the 189 with which the experiment was begun.

Autopsies of 5 healed animals, the tumors of which had been biopsied and diagnosed as malignant and which died 149, 129, 93, 49, and 19 days, respectively, after they had been pronounced healed, revealed neither macroscopic nor microscopic evidence of cancer.—Authors' abstract.

ROHDENBURG, G. L. [Lenox Hill Hosp., New York, N. Y.] THE EFFECT OF LOWERED ATMOSPHERIC PRESSURE ON SPONTANEOUS TUMORS IN MICE. Cancer Research, 1:310. 1941.

Lowering the atmospheric pressure, raising the temperature to 36° C., and placing the tumor-bearing mice in an atmosphere containing 10% carbon dioxide, 34% nitrogen, and 56% oxygen for periods up to 3 weeks did not cause either transplanted mouse sarcoma 180 or spontaneous mouse sarcoma to disappear.—Author's summary.

TALBOT, N. B., A. M. BUTLER, E. A. MacLACHLAN, and R. N. JONES. [Harvard Med. Sch., The Infants' and Children's Hosp., Boston, and Harvard Univ., Cambridge, Mass.] DEFINITION AND ELIMINATION OF CERTAIN ERRORS IN THE HYDROLYSIS, EXTRACTION, AND SPECTROCHEMICAL ASSAY OF α- and β- NEUTRAL URINARY 17-KETOSTEROIDS. J. Biol. Chem., 136:365-377. 1940.

In view of the diagnostic importance of variations in the urinary content of androgens, it is noteworthy that treatment of the crude neutral fraction of urinary extracts with Girard's reagent separates in good yield the androgenic fraction from the nonketonic fraction which contains the majority of interfering chromogenic substances. This purification largely eliminates the errors in the colorimetric assays of 17-ketosteroids by the Zimmermann reaction. Experimental details are given for the simultaneous hydrolysis and extraction of the urine and it is shown that this procedure is essential for accurate assays.—H. J. C.

ZEPHIROFF, P., and N. DOBROVOLSKAÏA-ZAVADSKAÏA. [Inst. du radium de l'Univ. de Paris] SUR UNE SUBSTANCE ANTISEXUELLE ISOLEE DE L'URINE D'UNE FILLETTE DE QUATRE ANS. [ANTI-ESTRUS SUBSTANCE ISOLATED FROM URINE OF FOUR-YEAR-OLD FEMALE.] Compt. rend. Soc. de biol., 133:405-407. 1940.

Fresh urine collected during 24 hours from this girl was precipitated with acetone. The sediment, suspended in a small quantity of acidulated distilled water, was centrifuged, the supernatant liquid was precipitated with 80% alcohol, and the precipitate dissolved in distilled water and centrifuged. One cc. of this solution corresponded to 100 cc. of urine. Tests on 2 series of adult female rats (125 gm. each) showed, in the 1st series, the capacity of this substance to stop the estrous cycle in young healthy females, and, in the 2nd series, its ability to retard development of the reproductive organs.—Authors' abstract.

ZEPHIROFF, P., and N. DOBROVOLSKAÏA-ZAVADSKAÏA. [Inst. du radium de l'Univ. de Paris] LE RÔLE PROBABLE DE L'ÂGE DANS LE FONCTIONNEMENT DE L'ÉPIPHYSE. [THE RÔLE OF AGE IN THE FUNCTION OF THE PINEAL GLAND.] Compt. rend. Soc. de biol., 134:57-58. 1940.

The product obtained from the urine of the girl mentioned above, when injected into immature rats, markedly retarded their growth; *viz.*, by 43% in males, by 37% in females. A similar preparation from the urine of an 8-year-old girl did not have this effect. Since this substance is probably of pineal origin, these experiments present new evidence indicating that age is a factor in the activity of the pineal gland.—Authors' abstract.

Cytology

FEKETE, E., O. BARTHOLOMEW, and G. D. SNELL. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] A TECHNIQUE FOR THE PREPARATION OF SECTIONS OF EARLY MOUSE EMBRYOS. Anat. Rec., 76:441-447. 1940.

The method described gives very satisfactory sections of mouse embryos up to 10 days of age. Accurate orientation of the embryo and certain differential staining are described.—Authors' abstract.

LEWIS, W. H. [Carnegie Inst. of Washington, Baltimore, Md.] MOTION PICTURES OF DIVIDING FIBROBLASTS. Anat. Rec., 76:91. (No. 2, Suppl. 1.) 1940.

Some of the visible features of cell division are explained in terms of viscosity changes of the plasmagel layer and of the spindle.—Author's abstract.

LEWIS, W. H. [Johns Hopkins Med. Sch., Baltimore, Md.] ON THE CHROMOSOMAL NATURE OF NUCLEOLI. Bull. Johns Hopkins Hosp., 66:60-64. 1940.

Studies of living fibroblasts in tissue cultures with the aid of motion pictures show that the larger nuceoli split up into chromosomes or parts of chromosomes during late prophase and that the large new nucleoli are formed by somewhat haphazard clumping of some of the chromosomal material in the daughter nuclei.—Author's abstract.

COMPARATIVE ONCOLOGY

BLAKEMORE, F., et al. DISCUSSION ON THE AETIOLOGY AND MORBID ANATOMY OF FOWL PARALYSIS

(NEUROLYMPHOMATOSIS). Proc. Roy. Soc. Med., 34:109-116. 1940.

This discussion, arranged by the Section of Comparative Medicine of the Royal Society of Medicine, was opened by F. Blakemore (paper published in full with references). Early experiments had shown the possibility of transmitting the disease in fowls by inoculation, but the proportion of successes was not high. Since other observations had indicated the importance of strain-resistance in neurolymphomatosis, it was decided to repeat the work using a different strain of fowl.

The strain employed was one in which the disease had been eliminated by inbreeding and isolation. The object of the experiments was to test the susceptibility of young chickens (1) by the intraperitoneal injection of emulsions of nerves and other tissues of affected fowls and (2) by contact with the naturally occurring disease. Judging by the occurrence of nerve lesions, fowl paralysis was transmitted to 23 of 78 injected chickens, and 14 of 33 placed in contact with naturally affected fowls became infected. Further experiments showed that the agent responsible was filterable, and that the disease could be transmitted by intravenous and intramuscular injection as well as by the feeding of infective material. Later, it was found that fowls over the age of 12 months were resistant to the infection. When large doses of material from acute passage cases were injected into 14 fowls varying in age from 2 to 5 months, the results were striking. Thirteen fowls developed paralytic symptoms from 36 to 225 days after injection. Marked nerve lesions, and infiltration of the liver and heart, were found on autopsy in each case.

Although the possibility was considered that the injected material merely stimulated a latent infection, the general trend of the results was regarded as being contrary to such a view. In the course of the work Blakemore was unable to confirm the findings of Patterson (J. Am. Vet. M. A., **41**:33. 1936.) that erythroleukosis, myeloid and lymphoid leukosis, are all expressions of the same transmissible disease. Nevertheless, the relationship of fowl paralysis to leukemia must be considered since the condition resembles Ellermann's lymphogenous leukemia in that both diseases are characterized by the development of lesions and the absence of leukemic changes in the blood.

The discussion was continued by R. E. Glover, H. P. Bayon, C. A. McGaughey, and L. E. Hughes. Glover pointed out that doubt still remained as to whether the disease should be regarded as neoplastic or inflammatory, or possibly as representing an intermediate stage between these two types. He regarded Blakemore's experiments, which he had been able to confirm, as most important, since there could now be no doubt that there existed certain forms of neurolymphomatosis which were infective and regularly inoculable.

Bayon recalled his own work (Proc. Roy. Soc. Med., **30**:377. 1937.) on the separation of a lymphoidal tumor into a leucotic condition and one producing a fibrosarcoma of the Rous type.—A. H.

TAYLOR, H. C., JR., and C. A. WALTMAN. [Memorial Hosp., New York, N. Y.] HYPERPLASIA OF THE MAMMARY GLAND IN THE HUMAN BEING AND IN THE MOUSE. MORPHOLOGIC AND ETIOLOGIC CONTRASTS. Arch. Surg., 40:733-820. 1940.

This systematic attempt to correlate and contrast benign breast hyperplasias includes clinical histories and histological sections of 103 cases of human chronic cystic mastitis and life histories and histologic sections of mammary glands and endocrine organs of 150 mice injected with various substances.—Authors' abstract.

WELLS, H. G., M. SLYE, and H. F. HOLMES. [Chicago Univ. Sch. of Med., Chicago, Ill.] THE OCCURRENCE AND PATHOLOGY OF SPONTANEOUS CARCINOMA OF THE LUNG IN MICE. Cancer Research, 1:259-261. 1941.

Of 147,132 mice coming to autopsy in the Slye laboratory, where every mouse is allowed to live out its span of life, 2,865 mice (2%) had lung tumors. Of these lung tumors, 104 (3.6%) showed metastases outside the lungs. All of these showed metastases in the mediastinal lymph nodes; many showed also growths along the chest wall and on the diaphragm. Only 10 showed metastases to other organs; namely, 5 in the kidney, 3 in the heart, 1 in the seminal vesicle, and 1 in the skull.

Of the 104 cases with metastases, 33 of the metastatic growths showed more or less the structure of a sarcoma, even when no sarcomatous elements were seen in the primary growth. Although in a number of primary lung tumors this sarcomatous structure was apparent, no primary sarcomas of the lung, without epithelial elements, have been observed in these mice. The mesenchymal or epithelial origins of these lung tumors is discussed.—Authors' summary.

CLINICAL AND PATHOLOGICAL REPORTS

Etiology

CRAMER, W. [British Cancer Commission, London, England] THE ETIOLOGY OF CANCER IN MAN IN THE LIGHT OF ANIMAL EXPERIMENTATION. Connecticut, M. J., 4:254-255. 1940.

The author states that in the light of recently acquired knowledge, the prevention of cancer is now theoretically possible. Cancer is not an autonomous growth, and the period of carcinogenesis in man requires from 10 to 50 years. Two main factors are involved in the development of cancer; namely, (1) the host factor, which depends upon the relative susceptibility of the patient, and (2) the carcinogenic factor. Hyperplastic changes (warts and epitheliomas) or hypoplastic changes (atrophy of gastric mucosa) in the tissues of the host may be the sites of cancer development. The nature of the carcinogenic agent is not definitely known. It may be a somatic change in the cell or a physiochemical change, but the agent is specific for the tissue. There is also a hormonal balance in the body, which, if upset, will result in cancer.

Susceptibility to the formation of cancer in particular tissues and organs is inherited by certain families. Cancer in males and the external group of cancers (skin, lung, tongue, and stomach) in general are relatively nonhereditary. The external cancers, however, are greatly influenced by social status, for the incidence is highest in the lowest social stratum, and vice versa. The author believes that education of the people in the lower economic levels will decrease the incidence of external cancers. He states, for example, that Vincent-Plummer's disease is a precursor of cancer of the esophagus. Treatment of the syndrome with

iron will prevent the malignancy. He emphasizes the importance of going back into the patient's history for many years to search for the first evidence of any abnormality, because here lies the future for the discovery of the precancerous conditions.—G. DeB.

GEHRMANN, G. H. [E. I. duPont de Nemours & Co.] INDUSTRIAL POISONS—PHYSIOLOGICAL AND CLINICAL CONSIDERATIONS. Connecticut M. J., 4:3-4. 1940.

It is the intermediate process of the manufacture of dyestuffs that is dangerous, the completed products not being seriously poisonous. Exposure to dyestuffs may cause 2 types of bladder tumor, papilloma and carcinoma. The average time required for their development is 11 years.—G. DeB.

Radiation—Diagnosis and Therapy

DALAND, E. M. [Massachusetts Gen. Hosp., Boston, Mass.] RADIATION DAMAGE TO TISSUE AND ITS REPAIR. Surg. Gynec. & Obst., 72:372-383. 1941.

Clinical study of damage from radiation. When the circulation of a region is poor the damaging effect from irradiation is greater, and after therapy the circulation becomes poorer so that ligation of vessels in that area is likely to produce gangrene. Cancer may develop in an area of irradiation dermatitis after a latent period as short as $2\frac{1}{2}$ years.—H. G. W.

KAMPERMAN, G. [Harper Hosp., Detroit, Mich.] THE PRESENT STATUS OF TREATMENT OF GYNECOLOGICAL CANCER. Surg., Gynec. & Obst., 72:384-390. 1941.

A statistical analysis of 854 cases of gynecological cancer treated in the Harper Hospital from 1922 to 1935, since the introduction of supervoltage roentgen therapy. In cancer of the cervix radiation constitutes the method of preference; and supplanting deep roentgen therapy with supervoltage roentgen therapy has brought up the 5 year survival to practically 35%. In carcinoma of the fundus the figure is approximately 50%, but no improvement has been obtained in carcinoma of the ovary.—H. G. W.

LENZ, M. [Presbyterian Hosp., New York, N. Y.] ROENTGEN THERAPY IN CANCER OF THE BREAST USED PREOPERATIVELY OR IN NON-OPERATED CASES. Surgery, 9:327, 1941.

Incurable cases do better with radiation alone.—A. M.

NEWELL, K. R. [Stanford, Calif.] R. S. STONE [Univ. of California Med. Sch.], and S. T. CANTREL [Seattle, Wash.] SYMPOSIUM ON METHODS OF IRRADIATION TREATMENT OF CARCINOMA OF THE CERVIX UTERI. Surgery, 9:328-329. 1941.

Stone suggested that surgery may be employed after irradiation therapy in Stage I cases, but he warned of a great margin of clinical error. Involved nodes occur in 25 to 33% of apparently operable cases; 5 to 10% have nodes without any evidence of parametrial spread. However, in 90% of Stage I cases the node involvement is limited to the primary lymph nodes. In Stages II, III, and IV external x-ray irradiation followed by radium within 2 weeks was advocated.—A. M.

ZAHL, P. A. [Memorial Hosp. New York, N. Y.] LOCALIZATION OF LITHIUM CONTAINING DYE IN CERTAIN TUMORS OF MICE, AFTER INTRAVENOUS INJECTION. Surgery, 9:327. 1941.

When the lithium atom captures a slow neutron it releases tremendous energies, far greater than the ionizing power of the electron. Slow neutrons are therefore being considered for therapy of "lithiated tumors." Tumors in mice show twice the lithium content of the normal surrounding tissue. The energy release in the tumor is theoretically three times that of normal tissue. Although of possible value in accessible cases the method offers a great many difficulties.—A. M.

Breast

DAVIS, H. H. [Omaha, Neb.] CYSTIC DISEASE OF THE BREAST. A CRITICAL REVIEW. Surgery, 9:295. 1941.

Cystic disease is attributed to an abundance of pituitary gonadotropic factor at a time when the ovarian factor is on the decline (35 to 50 years of age). The pure "cystic" variety is not important as far as any tendency to develop malignancy is concerned, but the "adenocystic" type is.—A. M.

EGGERS, C., DE CHOLNOKY, T., and JESSUP, D. S. D. [New York Post-Graduate Hosp., New York, N. Y.] CANCER OF THE BREAST. Ann. Surg., 113:321-340, 1941.

This is a general article reviewing the author's experience with 505 cases of breast cancer seen over a 10-year period (1924-33).

Radical mastectomy was performed in 253 cases. Preliminary electrosurgical excision of the lesion is advocated, followed by the Willy Meyer operation. Five-year arrests are reported for 55.6% of cases without axillary involvement and 21.2% for cases with positive lymph nodes.

The upper outer breast quadrant was the site of 48.6% of the tumors. The ulcerated group carry a particularly bad prognosis. The prognosis is better the smaller the tumor; a lesion under 2 cm. carries a 72.5% five-year salvage rate, as against 15.5% for one over 6 cm. As a rule the probability of axillary involvement increases with the size of the lesion; 83.3% of tumors over 4 cm. were associated with positive nodes and 100% over 8 cm. Nine per cent of cases without palpable nodes had axillary disease. The prognostic significance of delay is seen in the 76% salvage rate for cases treated within 1 month of the onset of symptoms, as against 20% for cases waiting over a year.

The same criteria for radical surgery are applied to cancer developing in the opposite breast. No case operated on for supraclavicular disease survived 5 years; half the cases died within a year. Preoperative irradiation is only employed for cases with a poor outlook; *e.g.*, the "inflammatory" type. Statistically, although not used routinely, postoperative irradiation seems to improve results; 38.7% five-year salvage as compared to 32.3% for the group.

A dozen tables are included with 8 photographs and 10 references.—A. M.

HINCHEY, P. R. [The Boston Dispensary, Boston, Mass.] NIPPLE DISCHARGE. A CLINICOPATHOLOGIC STUDY. Ann. Surg., 113:341-356. 1941.

According to the author, 3 types of lesions account for about 75% of all cases of nipple discharge: Carcinoma, papilloma, and the proliferative phase of cystic disease (adenosis).

The report is based on a series of 67 cases, of which 52 had biopsy confirmation. Including 2 cases of Paget's

disease in which discharge preceded any change in the nipple, carcinoma accounted for 24 of the cases (36%). This number represents 3.2% of a total of 742 breast carcinomas seen. In 1% of breast cancer cases, discharge may be an early sign and precede the appearance of a mass. Only about one-half these cases had a bloody discharge. The author warns against the diagnosis of benignity on the basis of a nonsanguinous discharge.

Chronic cystic disease accounted for 18 cases of nipple discharge (27%). Ten of 13 cases examined histologically showed epithelial hyperplasia, and 9 of these had a bloody discharge. Marked degrees of epithelial hyperplasia were associated with carcinoma in 6 of 22 cases. Such hyperplasia is regarded as a forerunner of malignancy.

Duct papilloma occurred in 10.5% of this group.

The chief problem is the discharging breast without palpable tumor. Since papilloma, a potentially malignant lesion, or "precancerous" epithelial hyperplasia, or even carcinoma may be present, the author advocates adequate biopsy and even simple mastectomy in the doubtful cases. A bibliography of 85 papers, 5 photomicrographs, and 3 tables are included.—A. M.

SOXENSON, F. [Copenhagen, Denmark] FIBROADENOMATOSIS OF THE BREAST. Surgery, 9:149. 1941.

Two hundred and fifty cases of fibroadenomatosis were seen. About 100 cases were treated with estrin. Over $\frac{1}{3}$ of the patients thus treated were free of symptoms. After 4 years no cases developed carcinoma. The therapeutic effect of estrin was found proportional to the amount of hyperplastic interlobular connective tissue.—A. M.

YOLTON, N., and C. REA. [Univ. of Minnesota, Minneapolis, Minn.] EXCRETION OF ANDROGENS AND ESTROGENS IN MALES WITH MAMMARY CARCINOMA. Proc. Soc. Exper. Biol. & Med., 45:54-55. 1940.

In 2 cases of mammary carcinoma in men 78 and 80 years old the range of urinary excretion of andogens in mgm. of androsterone in 24 hours was from 3.4 to 9.9; the range of excretion of estrogens in γ of estrone per 24 hours was from 0.8 to < 2.5. These values did not differ significantly from those of the controls. The low excretion of both androgens and estrogens in the aged, found by others, was confirmed. Twenty-four-hour urine specimens were extracted and fractionated according to the method of Gallagher, Peterson, Dorfman, Kenyon, and Koch. Assays for estrogens were made by the vaginal smear technic in mice. Androgenic assays were done by a modification of Zimmerman's method as described by Friedgood and Widden.—S. B-J.

Female Genital Tract

MORSE, A. H. [Yale Univ. Sch. of Med., New Haven, Conn.] PREGNANCY COMPLICATED BY CANCER OF THE CERVIX. A CASE REPORT. Connecticut M. J., 4:743-744. 1940.

This patient, in the 2nd month of gestation, was given the usual course of treatment for cancer of the cervix. No evidence of recurrence of the tumor was manifest 2 years and 9 months later.—A. DeB.

SMITH, F. R. [New York, N. Y.] NATIVITY AND CARCINOMA OF THE CERVIX. Surgery, 9:140. 1941.

A review of a portion of the information concerning the incidence of carcinoma of the cervix in the various nationalities of the population.—A. M.

HERR, E. A. [Waterbury, Conn.] ENDOMETRIOSIS. Connecticut M. J., 4:389-392. 1940.

The author discusses the history, etiology, pathology, etc. of this disease. That preservation of maximal ovarian function be the objective of therapy is stressed.—A. DeB.

ARENAS, N., R. SAMMARTINO, and A. L. PEPO. [Inst. de Maternidad, Prof. Ubaldo Fernandez] INTENSA LUTEINIZACIÓN FOLLICULAR DE ASPECTO TUMORAL EN UN EMBARAZO NORMAL. [PRONOUNCED FOLLICULAR LUTEINIZATION SIMULATING TUMOR IN A NORMAL PREGNANCY.] Rev. Asoc. méd. argent., 54:153-156. 1940.

According to the author this is the 2nd case so far observed, the first being reported in 1922 by Lévy-Solal *et al.* (Gynec. et Obst., **70**:5. 1922). The placenta did not show any signs of hydatidiform mole. Four photomicrographs and 3 photographs of the lesions are appended.—M. D-R.

GROSS, R. E. [Harvard Med. Sch., Boston, Mass.] NEOPLASMS PRODUCING ENDOCRINE DISTURBANCES IN CHILDHOOD. Am. J. Dis. Child., 59:479-628. 1940.

Various neoplasms which appear in children and produce endocrine disturbances; namely, granulosa cell carcinomas of the ovary, arrhenoblastomas, interstitial cell tumors of the testis, adrenal cortical tumors with symptoms of masculinization, basophilism, or feminization, tumors of the adrenal medulla, tumors of the pineal gland, and tumors of the floor of the third ventricle were briefly reviewed and discussed. Several detailed case reports were given, including history, physical, laboratory and pathological observations, surgical procedures, and postoperative course. One was a 13-month-old girl with a granulosa cell tumor with associated sexual precocity and high urinary estrogen (17,500 i.u. daily). Another case was a $3\frac{1}{4}$-year-old girl with an adrenal cortical carcinoma. This patient had an enlarged clitoris, pubic hair, deep voice, acne, boyish contour, advance skeletal age, hypertension, and showed a high urinary excretion of androgen (240 to 1,120 i.u. daily). A second masculinizing tumor in a 3-year-old boy was described. Two cases of adrenal tumors with clinical pictures resembling basophilism in 10- and 3-year-old girls were presented. One case of pineal neoplasm in a 10-year-old boy with typical findings of pubertus praecox was presented. Two cases, 1 female age 2 years, and 1 male age 8 years, showing sexual precocity, advanced skeletal development, and tumors (astrocytoma and ganglioneuroma) of the floor of the third ventrical were described in detail. The observations were stressed with reference to diagnosis of the focal organs of the primary disease, responsible for the clinical picture. Twenty-five illustrations and an extensive bibliography arranged according to type of neoplasm are included.—W. U. G.

HARRIS, F. I., and M. A. MEYER. [Mount Zion Hosp., San Francisco, Calif.] PLEURAL EFFUSION ASSOCIATED WITH OVARIAN FIBROMA (MEIGS SYNDROME). Surgery, 9:87-93. 1941.

This is the 16th reported case of benign ovarian fibroma associated with ascites and pleural effusion. The patient, 67 years of age, had a pelvic tumor for 6 years. Massive right pleural effusion cleared following removal of the ovarian fibroma. Roentgen therapy to the pelvis prior to operation did not influence either the size of the tumor or of the effusion. An x-ray, 1 gross and 2 microscopic photographs are included.—A. M.

KROCK, F. [Fort Smith, Ark.] ARRHENOBLASTOMA OF THE OVARY; FURTHER REMARKS. Surgery, 9:324. 1941.

The degree of virilism accompanying these tumors varies greatly and depends on the degree of differentiation of the tumor. The author reports a case with a return of femininity 6 weeks after surgery. Removal of a recurrence showed cartilage.—A. M.

LULL, C. B. [Philadelphia, Penn.] PUBERTAS PRAECOX DUE TO OVARIAN TUMORS. Surgery, 9:137. 1941.

Cases of 2 infants are reported, one with a granulosa cell tumor and one with an ovarian cyst at 20 months. Both reverted to normal after surgical removal of the tumors. The discussion dealt with malignant changes in granulosa cell tumors, failures of x-ray therapy in these cases, and the need of a national registry of ovarian tumors.—A. M.

BRINDLEY, G. V. [Temple, Texas] CARCINOMA OF THE FUNDUS OF THE UTERUS. A REPORT BASED ON 92 CASES. Surgery, 9:323. 1941.

The only accurate diagnostic method is carefully performed curettage. Two-thirds of the cases are of low grade malignancy. The best results were obtained with hysterectomy supplemented by x-ray or radium therapy; 79% remained well 5 years; 40% remained well with irradiation therapy alone.—A. M.

COSTOLOW, W. E. [Los Angeles Tumor Inst. Los Angeles, Calif.] TREATMENT OF UTERINE FIBROMYOMAS. J. A. M. A., 116:464-468. 1941.

An analysis of 986 patients with uterine fibromyoma treated between 1927 and 1937, with a follow-up period of from $2\frac{1}{2}$ to $12\frac{1}{2}$ years, with consideration of indications for radiation and results. In only 4 patients (0.4%) did cancer of the uterus develop after radiation therapy.—H. G. W.

SEARIGHT, W. [Univ. of Tennesee Med. Sch., Memphis, Tenn.] SARCOMA OF THE UTERUS. South M. J., 34:326-330. 1941.

Clinical discussion with emphasis on difficulty of early diagnosis.—H. G. W.

Male Genital Tract

HUGGINS, C., and C. V. HODGES. [Univ. of Chicago, Chicago, Ill.] STUDIES ON PROSTATIC CANCER. I. THE EFFECT OF CASTRATION, OF ESTROGEN AND OF ANDROGEN INJECTION ON SERUM PHOSPHATASES IN METASTATIC CARCINOMA OF THE PROSTATE. Cancer Research, 1:293-297. 1941.

In a group of 25 men with x-ray evidence of metastatic carcinoma to the bony pelvis, both acid and alkaline phosphatases were elevated in 19, only alkaline phosphatase was increased in 2, and both values were within normal limits in 4. In human prostatic cancer with marked elevation of acid phosphatase, castration or estrogen injection caused a prompt and sharp reduction of this enzyme to or towards the normal range while androgen injection caused this enzyme to increase. Acid phosphatase was present in trace amounts in cerebrospinal and spermatocele fluids at a time when the serum level was high.—Authors' abstract.

KICKHAM, C. J. E. [Pondville Hosp. Pondville, Mass.] DIAGNOSTIC PITFALLS IN CARCINOMA OF THE PROSTATE. J. Urol., 45:92-101. 1941.

Clinical study, with emphasis on the difficulty in diagnosis.—H. G. W.

McDONALD, J. R. and A. C. BRODERS. [Mayo Clinic, Rochester, Minn.] SEMINOMA AND TERATOMA IN THE SAME TESTIS. Am. J. Clin. Path., 11:138-144. 1941.

Case report with review of literature. The presence of 2 distinct tumors in the same testis suggests a separate histogenesis for the 2 types of neoplasm. The fact that typical neoplastic cells indicative of a seminoma could not be demonstrated in the teratoma, together with the absence of heterologous tissue in the seminoma, is added proof that these 2 neoplasms represent two different processes. Most of the reports of seminomas and teratomas in the same testis have occurred in men less than 35 years of age.—H. G. W.

Urinary System—Male and Female

DAVIDSON, B. [Beth Moses Hosp., Brooklyn, N. Y.] SPONTANEOUS REGRESSION OF A KIDNEY TUMOR. Urol. & Cutan. Rev., 45:13-15. 1941.

This is a case report of a 72-year-old male with clinical and radiological evidence of a left renal tumor. Operation revealed a large inoperable tumor but hemorrhage prevented biopsy. Three years later the patient was alive and well without any palpable mass. Death occurred 4 years after operation as a result of urinary retention and infection. No autopsy was performed.—A. M.

ERDMANN, J. F., H. A. BROWN, and H. W. SHAW. [New York Post-Graduate Hosp., New York, N. Y.] CHORIOEPITHELIOMA IN THE MALE OF EXTRAGENITAL ORIGIN. Urol. & Cutan. Rev. 45:1-6. 1941.

The case report is presented of a 45-year-old physician with an inoperable retroperitoneal chorioepithelioma and fibroadenoma of the breast. Autopsy disclosed a large pararenal mass involving adjacent lymph nodes and lungs. Microscopically, the tumor was composed of large blood sinuses lined by 2 types of cells resembling the Langhans and syncytial cells of the placenta. Twenty-four cases from the literature are tabulated. Ages ranged from 15 to 72. Six had gynecomastia. The author believes these tumors originate from embryonal rests of the urogenital folds. The Ascheim-Zondek test was negative in this case, although it is often positive. The paper is illustrated and includes 21 references.—A. M.

HENLINE, R. B. [New York Hosp., New York, N. Y.] FIBROSARCOMA OF A POLYCYSTIC KIDNEY WITH EXTENSION INTO THE VENA CAVA. Am. J. Surg., 51:408-413. 1941.

Report of a case with successful removal, despite opening of the vena cava.—H. G. W.

KEEN, M. R. and J. C. BERNSTEIN. [Huntington Hosp., Huntington, N. Y.] PRIMARY URETERAL CARCINOMA. Am. J. Surg., 51:402-407. 1941.

Case report.—H. G. W.

NYSTROM, G. [Borga, Norway] MALIGNANT TUMOR OF THE KIDNEY IN CHILDREN. Surgery, 9:148. 1941.

The author emphasizes the poor prognosis of kidney tumors in children. Three of his 4 patients died, the 4th is living a few months after operation.—A. M.

RILEY, A. [Boston City Hosp., Boston, Mass.] EPIDERMOID CARCINOMA OF THE PERINEAL URETHRA. Urol. & Cutan. Rev., 45:20-22. 1941.

Report of a case and a brief review of 100 cases of carcinoma of the male urethra which have been reported in the literature. They are most common near the junc-

tion of bulbous and membranous urethra, where strictures frequently follow urethritis. Squamous carcinoma is most frequent. Only 1 adenocarcinoma appeared in the group.—A. M.

Oral Cavity and Upper Respiratory Tract

ARBUCKLE, M. F. and A. C. STUTSMAN. [St. Louis, Mo.] PHARYNGEAL AND LARYNGOPHARYNGEAL CANCER. South. M. J., 34:219-221. 1941.

Preliminary report of 4 cases treated by a new combination of surgery and radiation therapy.—H. G. W.

BAXTER, H. [Montreal Children's Hosp., Montreal, Canada] A REVIEW OF MALIGNANT MELANOMA OF THE MOUTH. Am. J. Surg., 51:379. 1941.

Case report with analysis of 54 cases in the literature. It occurs much more often in men than in women, and in the majority of cases the patient is over 40 years of age. The maxilla is involved in 93% of the cases, and in 6 cases the patients were colored. The prognosis is bad.—H. G. W.

JACKSON, C. L. [Temple Univ. Med. Sch., Philadelphia, Penn.] THE TREATMENT OF CANCER OF THE LARYNX. South. M. J., 34:243-255. 1941.

Clinical discussion of indications for different forms of treatment.—H. G. W.

PERCY, J. F. [Los Angeles, Calif.] ADVANCED CANCER OF THE NOSE AND ACCESSORY SINUSES; TREATMENT BY ACTUAL CAUTERY. Surgery, 9:298. 1941.

The maxillary sinus is opened by a U-shaped incision over the sinus. The malignant tissue is destroyed by cautery. In some cases tumor was traced and treated in the brain substance itself.—A. M.

YANDO, A. H. and C. A. SCHLACK. [U. S. Naval Dental Sch., Washington, D. C.] ORAL CYSTS OF DENTAL ORIGIN. U. S. Nav. M. Bull., 39:22-26. 1941.

Four groups of cysts of dental origin are discussed. (1) Dentoperiosteal cysts, usually the result of traumatic or infectious stimulus, are called "dental root" or radicular cysts. They probably never become malignant. (2) Dentigerous cysts, containing unerupted hard structure of teeth, are referred to as "tooth-bearing" or follicular and are unilocular. Cystic and composite odontomas belong in this group. (3) Ameloblastoma and adamantinoma seem to be the result of ungoverned multiple enamel organ formation. Enamel is not formed, but a soft substance may form within the follicles as a result of degenerative processes. These multilocular cysts, although most common about the angle of the mandible, occur in the maxillar, posterior pharynx ("hypophyseal duct tumors"), and tibia. (4) Cysts of pseudodental origin are called median anterior maxillary or incisive canal cysts. The paper is illustrated by 10 figures.—A. M.

Intrathoracic Tumors—Lungs—Pleura

BRACHETTO-BRIAN, D., E. LASCANO, and O. CROXATTO. [Ramos Mejía Hosp., Buenos Aires] EL TERRENO ANATOMICO EN EL CANCER PRIMITIVO DE PULMÓN. [THE ANATOMICAL GROUND IN PRIMARY LUNG CANCER.] Rev. Asoc. méd. argent., 54:719-729. 1940.

Report based on the study of 40 cases of lung cancer, 95% occurring in men between 41 and 60 years old. Tobacco could be considered as an etiological factor in 51.8% and syphilis in 33.3% of the cases. The authors describe a severe and complex syndrome designated as "chronic sclero-mutilating bronchopneumonia with anthracosis and pleural adhesions" which occurred in 55% of the patients. In most cases cancer developed in the pulmonary lobe where these alterations were most pronounced. In another 20% of the cases there were important chronic bronchial lesions almost exclusive of the cancer, and in 7.5% there was congenital bronchiectasis. The authors conclude that lung cancer develops in an organ where extensive chronic lesions are present in 82.5% of cases. Microscopic precancerous lesions were metaplasia of the lining epithelia and proliferation of the glands of the bronchial mucosa. Nine photographs and 10 diagrams are included.—M. D-R.

CASTEX, M. R., E. S. MAZZEI, and M. S. DREYER. [Fac. of Med., Buenos Aires] CÁNCERES LATENTES DEL PULMÓN INICIADOS CLÍNICAMENTE POR METÁSTASIS ÓSEAS. [LATENT LUNG CANCER CLINICALLY INITIATED BY BONE METASTASES.] Rev. Asoc. méd. argent., 54:1-5. 1940.

Report of 2 cases in men, both heavy smokers and showing bronchitis of long standing. In one sciatic pain was the first symptom observed. It was not traced to a tumor in the 4th lumbar vertebra until 11 months later. Three months after the latter date pulmonary symptoms appeared, and a lung neoplasm was diagnosed. In the other the first symptom was swelling accompanied by pain in the shaft of the right fibula, both signs having started about 5 months before. A metastatic tumor was diagnosed at once but the primary lung cancer was not disclosed until a systematic radiological study of the patient was undertaken. The authors point out the radiological and clinical differences between primary and metastatic tumors in bones as observed in their cases. Radiologically they showed typical signs of bone destruction; there was no periosteal reaction, but a multiplicity of cavities in the same bone was noticeable; the neighboring joints were spared; and the signs of bone reaction, associated with those of bone destruction, had the aspect of cysts surrounded by a thick capsule. All these findings were confirmed at the post-mortem examination. Three pictures of the x-ray plates and 1 of the gross fibula lesion are appended.—M. D-R.

CHURCHILL, E. D. [Harvard Med. Sch. Boston, Mass.] RESECTION OF THE LUNG. Surgery, 8:961-991. 1940.

This comprehensive article summarizes the surgical experiences of the author and describes the technic he employs. The complications of lobectomy are discussed and the advantages of preoperative pneumothorax listed.

In the 10 year period before April, 1940, a diagnosis of primary lung tumor was made in 380 cases at the Massachusetts General Hospital. This diagnosis was confirmed by biopsy in 172 cases; 155 were primary carcinoma, 15 were adenoma (carcinoid). The author warns against endoscopic measures for eradication of even benign tumors. He advocates resection of tumor and lung segment by the intrahilar technic. Although pneumonectomy is the usual procedure for carcinoma, lobectomy is still advised for small peripheral tumors. Of the 155, 52 were operated on. Only 27 were resectable, with an immediate mortality of 55%. Of the group, only 5 were living and well. Six resections were done for metastatic cancer. This procedure

is still experimental. It is advocated for isolated pulmonary metastases discovered after eradication of the primary tumor. Ten anatomical diagrams are shown. There are 8 tables and a bibliography of 19 papers.—A. M.

CRAVER, L. F. [Memorial Hosp., New York, N. Y.] DIAGNOSIS OF MALIGNANT LUNG TUMORS BY ASPIRATION BIOPSY AND BY SPUTUM EXAMINATION. Surgery, 8:947-960. 1940.

Aspiration biopsy of the lung was begun at the Memorial Hospital in 1927. It has been gradually developed as a reliable method. In a series of 175 lung cancer cases biopsied during life, bronchoscopic examinations established the diagnosis in 80 cases and aspiration biopsy of the lung in 64. The procedure is only performed on cases in which the bronchoscopic approach fails to yield a positive biopsy. Good x-ray localization is necessary. Lipiodol is often of help. Fluoroscopic guidance is essential for small tumors. Two hundred and thirty lung punctures for biopsy were done with only 2 serious complications, attributed to air embolism.

Sputum examination was uniformly negative for cancer cells except in 2 cases where bits of actual tumor were coughed up.—A. M.

CURTIS, G. [Columbus, Ohio] MEDIASTINAL GANGLIONEUROMA. Surgery, 9:322. 1941.

Ganglioneuromas are usually found along the course of the sympathetic nerve trunks, in the neck, thorax, abdomen, and pelvis. Of 200 reported in the literature, some 30 were in the mediastinum. In the case reported, the tumor produced no symptoms. It was discovered by routine chest x-ray.—A. M.

ELIZALDE, P. I., and R. SAMMARTINO. [Inst. "Telémaco Susini," Buenos Aires] EL TERRENO ANATÓMICO EN EL CÁNCER PRIMITIVO DE PULMÓN. [THE ANATOMICAL GROUND IN PRIMARY LUNG CANCER.] Rev. Asoc. méd. argent., 54:481-487. 1940.

In 116 autopsies, the author studied 84 primary and 18 metastatic epitheliomas, and 9 primary and 5 metastatic sarcomas of the lung. In about two thirds of the cases different lesions, either separate or combined, were observed, and they were considered to be present long before the cancer developed. In 30 cases pleural adhesions either total or partial were found, and in the latter case they were always in the affected pulmonary lobe. Inflammatory processes were constantly encountered but in 9 cases they were chronic fibrous lesions of pneumonia and bronchopneumonia. Foci of pleurisy (3 cases), bronchiectases (3 cases), sclerosis and chronic emphysema (27 cases) of very old standing were also found. In 16 cases the tumor arose from an irregularly shaped strongly anthracosic scar, present either in the lung or in the pleura. Detailed histories of 11 cases and as many illustrations of gross or microscopic preparations are appended.—M. D-R.

GOLDMAN, A. and H. B. STEPHENS. [Univ. of California Med. Sch., San Francisco, Calif. POLYPOID BRONCHIAL TUMORS. J. Thoracic Surg., 10:327-347. 1941.

Emphasizing the differentiation of bronchial adenomas from carcinomas. Three morphologic types of adenomas occur: endobronchial, intramural, and extra-endobronchial, the incidence of the two latter being greater than previously reported. Endoscopic removal of bronchial adenomas is indicated to re-establish bronchial drainage, but sooner or later pulmonary resection is indicated.—H. G. W.

GREAVES, F. C. [Med. Corps, U. S. Navy] LUNG CYSTS. U. S. Nav. M. Bull., 38:308-311. 1940.

A case report is presented. The patient, a 70-year-old medical officer, died of papillary renal carcinoma with metastases to liver, ribs, and abdominal nodes. Both lungs contained multiple cysts. He had had 3 attacks of spontaneous pneumothorax in 37 years.—A. M.

HALPERT, B. [Louisiana State Univ. Sch. of Med., New Orleans, La.] MORPHOLOGIC ASPECTS OF CARCINOMA OF THE LUNG. Surgery, 8:903-909. 1940.

Between January, 1931 and June, 1939 there were 92 autopsied cases of carcinoma of the lung at the Charity Hospital of Louisiana at New Orleans. During that period necropsies were performed on 169 cases of gastric cancer.

The distribution of cases among white and colored patients was about equal. There were 6 women in the group (86:6); only one was colored. Ages ranged from 21 to 75. Seventy-seven per cent were in the 6th decade or older. The right lung was involved more frequently (49:38). About 50% of the lesions were accessible to bronchoscopic visualization.

The concept is repeated that the "reserve cell" is the parent cell of all lung carcinomas. Cellular structure reveals 3 main types. These are seldom pure. The squamous cell variety composes over 50% of the cases. The columnar cell type presents acinar, tubular, and papillary forms; it represents 20% of the cases. The reserve cell carcinoma follows no pattern; it composes the rest of the group. The microscopic appearance cannot be predicted from the gross specimen. About 90% of the cases had regional node involvement. Sixty-eight per cent had distant metastases in this order: liver (30%), suprarenal (15%), kidney (15%), skeleton (10%). Six photomicrographs and 7 references are included.—A. M.

HEUER, G. J. [Cornell Univ. Med. Sch. New York, N. Y.] SURGICAL TREATMENT OF TUMORS OF THE MEDIASTINUM. Ann. Surg., 113:357-363. 1941.

The author briefly mentions the extraordinary variety of tumors found in the mediastinum and discusses their general symptomatology, treatment, and prognosis.

Between September, 1932 and November, 1939, 199 mediastinal lesions were seen at the New York Hospital; 145 were neoplastic. Of these, 47 belonged to the lymphoma group; 29 were cases of Hodgkins disease and 18 of lymphosarcoma. There were 17 primary malignant tumors, including 5 teratomas, 3 each of neurogenic sarcoma, liposarcoma, and superior sulcus carcinoma, 2 malignant thymomas and 1 fibrosarcoma. Of the secondary malignant tumors, 31 originated in the bronchi, and 12 elsewhere. Thirty-nine were benign tumors, including 5 each of dermoids, neurogenic tumors, chondromas or chondromyomas, 3 thymomas, etc.

Twelve malignant cases were explored. In 6 the lesion was removed with 2 deaths. One of the surviving 4 died within a year, the other 3 are living and well up to 2 years. Seventeen benign cases were explored with removal of the tumor in 16. Of the 13 survivors, 12 are living and well. In the fatal case a chondroma recurred and metastasized.—A. M.

HOLINGER, P., and D. B. RADNER. [Univ. of Illinois Coll. of Med. and St. Luke's Hosp., Chicago, Ill.] BRON-

CHOSCOPIC DIAGNOSIS OF BRONCHIAL CARCINOMA. Surgery, 8:939-946. 1940.

The authors emphasize that there is an actual increase in bronchiogenic carcinoma. Symptoms and methods of diagnosis are discussed. A bibliography of 31 papers is listed.—A. M.

HOLST, J. [Oslo, Norway] MEDIASTINAL TUMORS. Surgery, 9:155-156. 1941.

The author operated on 11 cases of mediastinal tumors and benign peripheral lung tumors without any mortality. The technic is described.—A. M.

KELLEY, C. H. [Provident Hosp., Chicago, Ill.] PRIMARY CARCINOMA OF THE LUNG: REPORT OF TWO CASES. Radiology, 35:61-64. 1940.

Report of 2 cases proved by bronchoscopic biopsies and necropsies.—E. A. L.

KORNBLUM, K. and H. H. BRADSHAW. [Jefferson Med. Coll., Philadelphia, Penn.] INTRATHORACIC NEUROFIBROMAS. Surgery, 9:330. 1941.

These tumors are constantly posterior, close to the spine. They do not respond to x-ray and should be extirpated.—A. M.

MAHER, P. P. and A. H. STADERMAN. [Med. Corps, U. S. Navy] BRONCHOGENIC CARCINOMA. A REVIEW OF THIRTY VERIFIED CASES. U. S. Nav. M. Bull., 38:541-553. 1940.

Between January 1, 1937 and August 1, 1939 there were 15,529 in-patients at U. S. Naval Hospital in Philadelphia. Among 369 autopsies performed in 789 cases, 128 were disclosed to be cancer verified by biopsy or necropsy. Carcinoma of the colon (21.8%), bronchogenic carcinoma (18.7%), and gastric carcinoma (14.1%) were the most frequent.

Thirty verified cases of bronchogenic carcinoma are reviewed and symptoms described. Their average age was 50 years. About 56% occurred in the right lung and 40% in the left.

In 17 autopsied cases of lung carcinoma the principal areas of metastases were: hilar nodes (53% of cases), mediastinal nodes (53%), kidneys (41%), liver (35%), brain (29.5%), skeleton (24%), adrenals (23.6%). Small bronchogenic carcinomas arising in secondary bronchi may be undifferentiated and metastasize widely with few clinical symptoms. About 36% of the cases were squamous carcinoma, 20% adenocarcinoma, 43.3% undifferentiated. Twelve tables and 6 roentgenograms are included.—A. M.

MARANO, A., A. CARDEZA, and R. H. MATERA. [Durand Hosp., Buenos Aires] CONSIDERACIONES ANATOMOPATOLÓGICAS SOBRE 5 CASOS DE ASOCIACIÓN DE CÁNCER Y TUBERCULOSIS PULMONAR. [PATHOLOGICAL CONSIDERATIONS OF 5 CASES OF ASSOCIATION OF CANCER AND PULMONARY TUBERCULOSIS.] Rev. Asoc. méd. argent., 54:713-717. 1940.

In 3 cases both processes were found in the same lung and the same lobe while in the 2 others each process was in a different lung. The gross and histological types of the tumors are described. There were no extrapulmonary tubercular lesions thus confirming the rule that cancer cachexia (very pronounced in all the 5 cases) does not favor the multiplication of the Koch bacillus. Despite the exudative type of the tubercular lesions they did not show the activity and invasiveness which would usually be found, the picture being dominated both clinically and histologically by the neoplastic disease. The authors believe that both diseases developed independently. Five illustrations are appended.—M. D-R.

MOORE, S. [Washington Univ. Sch. of Med., St. Louis, Mo.] BODY-SECTION RADIOGRAPHY IN MALIGNANCY OF THE LOWER RESPIRATORY TRACT. Surgery, 8:924-938. 1940.

In 15 years the Chest Service of Washington University Medical School has seen 400 cases of bronchiogenic carcinoma. Its incidence is placed at between 5 to 10% of all cancers. It is about as common as cancer of the rectum.

This paper is copiously illustrated with 24 x-ray photographs comparing the conventional films with those taken by "body-section radiography." This procedure, best suited for the respiratory tree, is of maximum value where superimposition of structures shows a minimum of contrast in the conventional film. This is especially true of the upper lobes, inaccessible to bronchoscopy. Mediastinal investigation, done by lateral views, is particularly valuable.—A. M.

MOSTO, D. [Fac. of Med., Buenos Aires] LAS ALTERACIONES BRONCO-PULMONARES CONCOMITANTES EN EL CÁNCER DE PULMÓN. [BRONCHO-PULMONARY ALTERATIONS ACCOMPANYING LUNG CANCER.] Rev. Asoc. méd. argent., 54:437-465. 1940.

In this extensive review the author analyzes the relation between lung cancer and the following factors in the same organ: (a) congenital lesions; (b) metabolic alterations; (c) proliferative lesions of the connective tissue (fibrils and collagen, elastic tissue and reticulum); (d) metaplasia of bronchial and alveolar cells; (e) lesions in vessels, lymphoid nodules, and muscular and nervous structures; (f) chronic circulatory disturbances (atelectasis, congestion, edema); (g) inflammation (pneumonia and bronchopneumonia, alveolitis, suppurative lesions, tumoral pneumonia of Pepere and Fabris, influenza, tuberculosis, syphilis, mycoses, parasitic lesions); (h) modifications of position, expansion, and continuity (emphysema, atelectasis); (i) pneumoconiosis; (j) bronchial lesions; (k) pleural lesions; (l) other lung tumors. The study is based on clinical and experimental observations by other authors (300 references are appended) as well as on 79 personal cases. The author divides the lesions accompanying lung cancer as pre-existing to, simultaneous with, and consecutive to the cancer, and it is possible to determine with precision the chronology of such lesions. Most of them are the consequence of the growth although in some cases the tumor develops from pre-existing lesions. As far as is known none of the factors reviewed can be considered as playing a part in the etiology of lung cancer. All known alterations of the respiratory system can coexist with lung cancer. There is no antagonism between lung cancer and any other pulmonary lesion.—M. D-R.

NYSTROM, G. [Upsala, Sweden] SUCCESSFUL PNEUMONECTOMY FOR REMOVAL OF METASTATIC CARCINOMA. Surgery, 9:155. 1941.

The patient, aged 61, had a carcinoma of the uterus removed 10 years before. Pneumonectomy was done for a metastatic carcinoma the size of a fist.—A. M.

OCHSNER, A. and M. DEBAKEY. [Sch. of Med., Tulane University, New Orleans, La.] PRIMARY CARCINOMA OF THE LUNG. New Orleans M. & S. J., 93:387-394. 1941.

General considerations.—H. G. W.

OCHSNER, A., and M. DEBAKEY. [Sch. of Med., Tulane Univ., New Orleans, La.] SURGICAL CONSIDERATIONS OF PRIMARY CARCINOMA OF THE LUNG. Surgery, 8:992-1023. 1940.

At the Charity Hospital in New Orleans during the past year there were more necropsies for lung carcinoma than for gastric cancer. All statistical data point to a phenomenal rise in the incidence of the disease. The author advocates pneumonectomy in operable cases as the only rational procedure for malignant disease of the lung.

The number of total pneumonectomies reported has doubled in less than 2 years. The authors discuss the collected statistics of 158 cases. Males were 3 times as numerous as females; 18.5% were below 40; 70.4% were in the 5th and 6th decade. About 25% of the cases occurred in the right lower lobe.

Mortality figures are slightly higher for right-sided lesions. Intrahilar ligation of structures results in a 38.3% mortality; mass ligation carries a 75.8% mortality. After operation, 83 cases recovered; 67 have available follow-up data. Forty-seven are living; only 3 are living and well 5 years after operation.

Fourteen diagrams illustrate the technic of the operation. Three x-rays and 2 gross pathological photographs are shown. There are 10 statistical graphs and a bibliography of 162 papers.—A. M.

POLAK, M. [Rawson Hosp., Buenos Aires] LA METAPLASIA DEL EPITELIO BRONQUIAL Y SU RELACIÓN CON EL CARCINOMA DEL PULMÓN. [METAPLASIA OF BRONCHIAL EPITHELIUM AND ITS RELATION TO LUNG CANCER.] Rev. Asoc. méd. argent., 54:489-493. 1940.

Inflammatory processes bring about stratification of the bronchial epithelium with infiltration of the submucosa, but this lesion can hardly be called metaplasia (as defined by Orth) as Askanazy, Huguenin, *et al.,* have done, nor can it be regarded with certainty as a precancerous lesion. The method of silver impregnation of Rio Hortega always shows the presence of an intact basal membrane. On the other hand, in 79 cases of bronchial cancer, true metaplasia (without infiltration) was observed in only 2 cases while stratification was commonly observed.—M. D-R.

RADICE, J. C. [Rawson Hosp., Buenos Aires] EL ESTADO DEL RETÍCULO, DEL COLÁGENO Y FIBRAS ELÁSTICAS DEL PULMÓN CON CÁNCER PRIMITIVO. [THE RETICULUM, COLLAGEN, AND ELASTIC FIBERS IN PRIMARY LUNG CANCER.] Rev. Asoc. méd. argent., 54:543-547. 1940.

The elastic tissue resists neoplastic invasion although it is displaced and split by the growth. There is a ring-like hyperplasia of the elastic tissue in the blood vessels, especially when the latter are compressed. The collagen is always increased surrounding the advanced edges of the growth. It is also increased around the bronchi and sometimes in the alveoli. The reticulum is condensed in the neoplasm giving an impression of a false capsule. It is also increased around the zones of necrosis. Six photomicrographs are included.—M. D-R.

SCHLOSSBERG, R. [Rawson Hosp., Buenos Aires] LAS ALVEOLITIS EN EL CÁNCER DE PULMÓN. [THE ALVEOLITIS OF LUNG CANCER.] Rev. Asoc. méd. argent., 54:541-542. 1940.

A description of the process (tumor pneumonia of Pepere-Fabris) considered specific for lung cancer. Five photographs are appended.—M. D-R.

SINGER, J. J. [The Cedars of Lebonan Hosp., Los Angeles, Calif.] PRIMARY BRONCHIOGENIC CARCINOMA. A CLINICAL STUDY. Surgery, 8:910-923. 1940.

All statistics given are quoted. Only 2 original cases are presented. The author describes a thoracoscope with a universal adaptor placed on the end of a small canula with 2 openings. This permits biopsy, coagulation, aspiration, etc., under direct vision. A photograph of the instrument is shown. Four x-rays, a photomicrograph and 11 references are included.—A. M.

STEIN, J. J. [Edward Hines Jr. Hosp., Hines, Ill.] CARCINOMA OF THE LUNG. A CLINICAL STUDY. U. S. Nav. M. Bull., 38:329-334. 1940.

The author finds that carcinoma of the lung is increasing. The reported incidence varied from 2.6% to 19.3% of all carcinomas. At the Hines Hospital 7 cases were seen in 1931 and 57 cases in 1937, representing 1.7% and 7.3%, respectively, of all cases.

A review of 768 cases from the literature shows 85% males. Approximately 60% are between 40 and 60 years of age. Seventy-five per cent involved a first order bronchus; 70% involved the right lung. All probably arise from the basal epithelium of the bronchus. The three outstanding early symptoms are cough, chest pain, and dyspnea. Irradiation therapy is palliative.

Superior pulmonary sulcus tumors usually arise in terminal bronchioles. Ninety-five per cent of cases are in men. Irradiation rarely relieves symptoms. These tumors often produce unilateral shoulder pain radiating down the arm, weakness of the arm, Horner's syndrome, and an apical lung shadow.—A. M.